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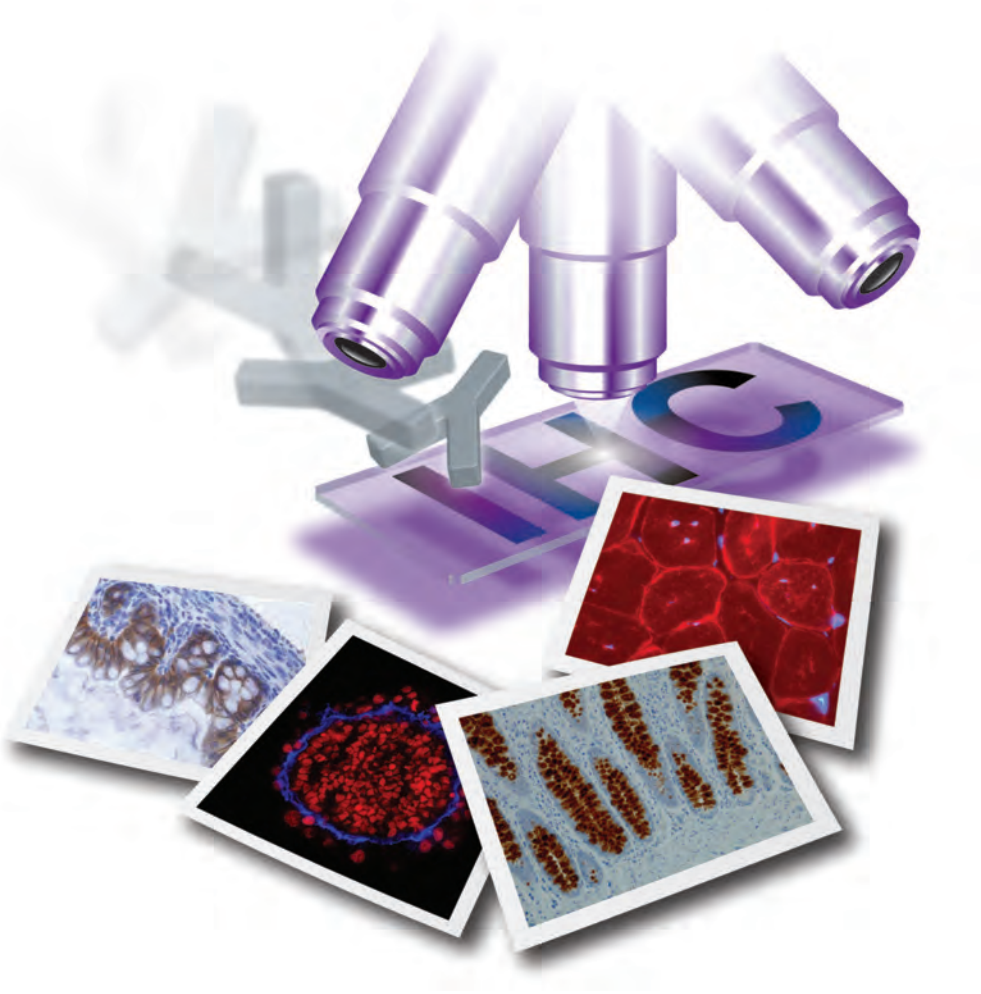
Immunohistochemistry (IHC) an Application Guide



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Tips for designing a successful IHC experiment

Immunohistochemistry (IHC) is a method for demonstrating the distribution and location of proteins in tissue sections. Though less sensitive quantitatively than immunoassays such as western blotting or ELISA, it enables the observation of processes in the context of intact tissue. This is especially useful for assessing the progression and best treatment options of diseases such as cancer. In general, the information gained from IHC combined with microscopy provides a valuable perspective that can help make sense of data obtained using other methods.

Immunohistochemical staining is accomplished with antibodies that recognize the target protein. Since antibodies are highly specific, the antibody will bind only to the protein of interest in the tissue section. The antibody-antigen interaction is then visualized using either chromogenic detection, in which an enzyme conjugated to the antibody cleaves a substrate to produce a colored precipitate at the location of the protein, or fluorescent detection, in which a fluorophore is conjugated to the antibody and can be visualized using fluorescence microscopy.

Although IHC is a relatively straightforward experimental method there are a number of variables that have to be identified and optimized for each individual IHC study. Optimization and standardization of these variables would allow consistent and reproducible results. Some variables that should be considered are given in Table 1.

Table 1. Immunohistochemistry variables

Variable	Factors to consider
Antigen	Species, expression levels, sample types
Epitope	If mapped – dependence on post-translational modification
Appropriate Controls	Positive & negative controls - no primary antibody, isotype control, absorption control, tissue type control
Sample preparation	Fixed or frozen
Fixation Method	Perfusion or immersion (with our without freezing)
Fixative	Formaldehyde, alcohols or acetone (including concentration, pH, temperature, incubation time and diluents)
Blocking Reagent	Normal serum (serum species is critical depending on secondary antibody), bovine serum albumin, gelatine or non-fat milk, commercial blocking buffers
Antigen Retrieval	Proteolytic-induced Epitope Retrieval (PIER) or Heat-induced Epitope Retrieval (HIER)
Detection Method	Direct or indirect (with or without amplification)
Detection Complex	ABC, LSAB, polymer or micro-polymer
Primary Antibody	Monoclonal or Polyclonal
Secondary Antibody	Species and label
Labelling Method	Chromogenic/enzymatic or fluorescence
Label	Fluorochromes and spectral properties
	Chromogens: 3,3 Diaminobenzidine DAB,3-amino-9-ethylcarbazole (AEC), benzidine dihydrochloride (BDHC), 3,3',5,5'-tetramethylbenzidine (TMB), New Fuchsin, 5-bromo-4-chloro-3-indolyl-phosphate/nitro blue tetrazolium (BCIP/NBT)
Counterstain	Fluorescence: DAPI, DRAQ7™, Nuclear green
	Chromogenic: hematoxylin and eosin or special stain
Mounting Reagent	Fluorescence: anti-fade mounting medium
	Chromogenic: organic/aqueous mounting medium
Visualization & Analysis	Fluorescence microscope or standard microscope. Analysis by eye or software solutions

Sample preparation and Reagents for sample preparation

Sample preparation is an essential and key step in successful histological techniques. Ensuring appropriate and good sample preparation contributes to producing good quality IHC staining.

Fixation

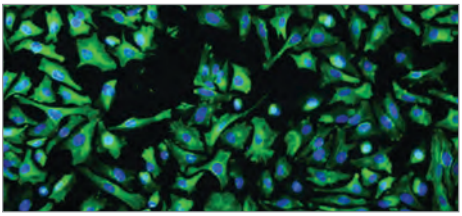
Fixation prevents the autolysis and necrosis of excised tissues, preserves antigenicity, enhances the refractive index of tissue constituents and increases the resistance of cellular elements to tissue processing. Tissue processing includes dehydration, clearing of dehydrating agents, infiltration of embedding media, embedding and sectioning of tissues. The choice of fixative to be used is dependent on the antigen, it is valuable to try and maintain standardized fixation conditions in order to produce reproducible staining. A robust and optimized fixation protocol is a critical step in an immunohistochemistry protocol as an antigen that has been inappropriately fixed may not be detected in downstream detection. Some guidelines for the type of fixative to use are given in Table 2.

Table 2. Fixation guidelines (Fixatives used for a given antigen)

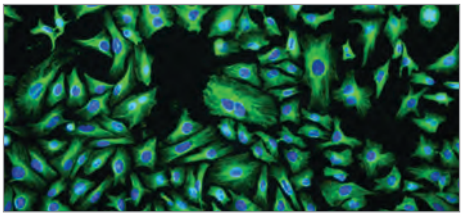
Antigen	Fixative
Most proteins, peptides and enzymes of low molecular weight	Cells/Cytological preparations: 4% Formaldehyde Tissue sections: 10% Neutral-Buffered Formalin (NBF)
Delicate tissue	Bouin's Fixative
Small molecules such as amino acids	4% Formaldehyde
Blood-forming organs (liver, spleen, bone marrow); connective tissue	Zenker's Solution Helly Solution
Nucleic acids	Carnoy's Solution
Large protein antigens (immunoglobulin)	Ice-Cold Acetone or Methanol (100%)
Nuclear morphology	Zinc formalin
Ideal for electron microscopy	4% Formaldehyde-1% Glutaraldehyde

Figure. 1: ICC Example of how choice of fixative affects immunostaining patterns:

Formaldehyde



Methanol



Primary: ab7291, mouse monoclonal [DM1A] to alpha-Tubulin, 5ug/ml
Secondary: ab150105, donkey anti-mouse IgG Alexa Fluor® 488, 2ug/ml

Embedding

Experimental variables and potential downstream protocols influence the most appropriate method that should be used for sample embedding; in addition some epitopes may not survive fixation or embedding. Embedding of tissue is important in preserving morphology and giving the tissue support during microtomy. Some guidelines for tissue embedding are given in Table 3.

Table 3: Embedding guidelines

	Paraffin embedded	**Frozen tissue**
Fixation	Pre-embedding	Pre/post-sectioning
Sectioning	Microtome	Cryostat
Storage	Multiple years at room temperature (*Note:* antigen may change over time)	1 year at -80°C (longer at -190°C)
Advantages	Preserves tissue morphology	Preserves enzyme & antigen function
Limitations	Overfixation can mask the epitope	Formation of ice crystals may negatively affect tissue structure
Downstream protocols	DNA and RNA for PCR amplification (extensive crosslinking prevents extraction of long nucleotide strands, free nuclei for ploidy and cell cycle analysis, cell for flow cytometry)	DNA, RNA, free nuclei for FISH or cell cycle analysis
Precautions	Duration and intensity of tissue heating should be kept to a minimum as melting temperature of paraffin wax (50-60°C) can be deleterious to staining of some antigens	Tissues should not be frozen slowly to prevent formation of ice crystals and tissues should be allowed to warm to cutting temperature (-20°C) in cryostat to avoid shattering

Tissue treatment

Antigen retrieval

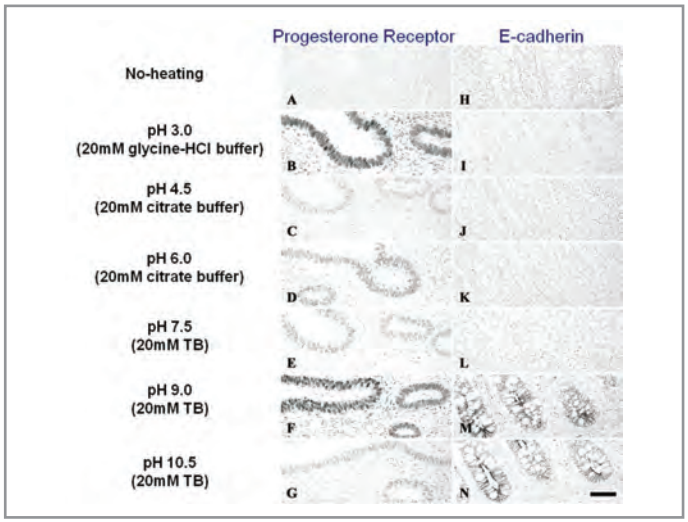
The process of sample fixation can lead to cross linking which masks epitopes and can restrict antigen antibody binding. Such masked epitopes can be retrieved using Proteolytic-induced Epitope Retrieval (PIER) or Heat-induced Epitope Retrieval (HIER). In the PIER method enzymes such as Proteinase K, Trypsin and Pepsin are used to restore the binding of an antibody to its epitope. The HIER method utilizes heat from a variety of sources (microwave, pressure cooker, steamer, waterbath or autoclave) to unmask epitopes. The preferred technique for optimal retrieval is dependent on tissue, fixation and/or primary antibody and must be optimized by the histologist. Some antigens can be more efficiently retrieved by a combination of heating and enzyme digestion (e.g. some cytokeratins and immunoglobulin light chains). An initial recommendation for optimization is to test two methods from HIER such as citrate buffer (pH 6) and Tris-EDTA (pH 9) and one or two methods from PIER such as Proteinase K and/or Trypsin. Some suggested guidelines are given in Table 4 but conditions must be optimized for each antigen.

Table 4. Epitope retrieval guidelines

	HIER	PIER
Advantages	Gentler epitope retrieval and more definable parameters	Useful for difficult to retrieve epitopes
pH	Citrate buffers of pH 6 are widely used but high pH buffers have been demonstrated to be widely applicable for many antibodies. Optimal pH must be determined in the lab	Typically 7.4
Recommended antigens	No specific antigens	Cytokeratins and immunoglobulins
Temperature	Approximately 95˚C	Typically 37˚C
Incubation time	10-20 minutes (20 minutes is common)	5-30 minutes (10-15 minutes is common)
Buffer composition	Depending on pH required as pH is target dependent (as shown in Figure 2). Popular buffer solutions include Sodium citrate, EDTA and Tris-EDTA	Neutral buffer solutions of enzymes such as pepsin, proteinase K or trypsin
Precautions	The use of heating methods such as microwaves can result in unbalanced epitope retrieval due to hot and cold spots. Rigorous boiling can also lead to tissue dissociation from the slide	Enzymatic retrieval can sometimes damage the morphology of the section – concentration and treatment need optimization

Note: Antigen retrieval may not be required for frozen sections as cross links are formed through formalin fixation.

Figure 2. Effects of pH on heat-induced antigen retrieval in human tissues



Emoto et al. (2005) Mechanisms of Heat-induced Antigen Retrieval: Does pH or Ionic Strength of the Solution Play a Role for Refolding Antigens? J Histochem Cytochem 53 (11):1311-21. Fig. 3)

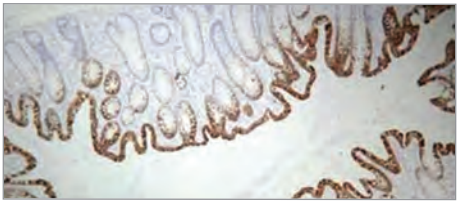
Buffers and epitope retrieval reagents

IHC Buffers

Product name	Size/description	Product code
Background Reducing Buffer	50mL	ab64234
10x Citrate Buffer pH 6.0	125mL	ab64214
100x Citrate Buffer pH 6.0	50mL	ab64236
10x EDTA Buffer pH 8.0	125mL	ab64216
100x EDTA Buffer pH 8.0	50mL	ab64239
10x PBS buffer	1L	ab128983
25x PBS Buffer pH 7.6	125mL	ab64026
20x PBS Buffer with Tween 20	125mL	ab64028
25x PBS Buffer pH 7.6	1L	ab64246
20x PBS buffer with Tween 20	1L	ab64247
25x TBS pH 7.4	125mL	ab64203
20x TBS with Tween 20	125mL	ab64204
25x TBS pH 7.4	1L	ab64248
20x TBS-T with Tween 20	1L	ab64250
10x Tris Buffer pH 10.0	125mL	ab64222
100x Tris buffer pH 10.0	50mL	ab64251
Tween 20	50mL	ab128987

Epitope recovery/tissue pretreatments

Product name	Size/description	Product code
Antigen Retrieval Buffer (100X Citrate Buffer pH 6.0)	125mL	ab93678
Antigen Retrieval Buffer (100X Citrate Buffer pH 6.0)	250mL	ab94674
Antigen Retrieval Buffer (100X EDTA Buffer, pH 8.0)	125mL	ab93680
Antigen Retrieval Buffer (100X EDTA Buffer, pH 8.0)	250mL	ab94677
Antigen Retrieval Buffer (100X Tris Buffer, pH 10.0)	125mL	ab93682
Antigen Retrieval Buffer (100X Tris Buffer, pH 10.0)	250mL	ab94680
Antigen Retrieval Buffer (100X Tris-EDTA Buffer, pH 9.0)	125mL	ab93684
Antigen Retrieval Buffer (100X Tris-EDTA Buffer, pH 9.0)	250mL	ab94681
Heat Mediated High pH Antigen Retrieving Solution	1L	ab972
10x Heat Mediated Antigen Retrieval Solution pH 6.0	250mL	ab973
10X Tris-HCl Buffer for HIER	50mL	ab128986
HistoReveal	15mL	ab103720
Pepsin Solution	7mL	ab64201
Pepsin Solution	15mL/125mL	ab128991
Proteinase K	4mL	ab64220
Trypsin Enzymatic Pretreatment	15mL or 125mL	ab128214
Trypsin Enzymatic Pretreatment Kit	7mL	ab64205
Trypsin Enzymatic Antigen Retrieval Solution	1.6mL concentrated liquid trypsin and 25ml trypsin buffer	ab970



Featured Product:

HistoReveal (ab103720)

HistoReveal (ab103720) gives optimal revelation of target antigens, allowing the use of less primary antibody and giving superior staining at the same time. Image: Cytokeratin 20 being stained in colon tissue (Formalin/PFA-fixed paraffin-embedded sections) following antigen retrieval using HistoReveal.

Permeabilization

Permeabilization is only required when the antibody needs access to the inside of the cells to detect the protein. These include intracellular proteins and transmembrane proteins whose epitopes are in the cytoplasmic region. Solvents or detergents are typically used for permeabilization.

Solvents: Can be used after fixation with crosslinking agent e.g. paraformaldeyhyde. Recommended for cytoskeletal, viral and some enzyme antigens.

Detergents: Much milder and will not dissolve plasma membrane, suitable for antigens in the cytoplasm or the cytoplasmic face of the plasma membrane and for soluble nuclear antigens.

Table 5. Solvent and detergent guidelines

	Solvents	Comments
Solvents	Acetone	Fixation will also permeabilize.
	Methanol	Fixation can be used to permeabilize but is not always suitable.
Detergents	Triton or NP-40	Use 0.1 to 0.2% in PBS for 10 min only.
	Tween 20, Saponin, Digitonin and Leucoperm	Use 0.2 to 0.5% for 10 to 30 min.

Blocking

Protein on protein blocking

Blocking with sera is essential to block unspecific absorbance to tissue or to Fc receptors. Using a serum matching the species of secondary antibody is recommended. When performing multiple stains using secondary antibodies from different species it may be necessary to using blocking serum from the species of both secondary antibodies.

Blocking Sera

Sterile sera

Product name	Description/size	Product code
Bovine Calf Serum (sterile)	25mL	ab138477
Cat Serum (sterile)	50mL	ab139511
Chicken Serum (sterile)	25mL	ab138577

Product name *(continued)*	Description/size	Product code
Dog Serum (sterile)	50mL	ab7476
Donkey Serum (sterile)	50mL	ab138579
Goat Serum (sterile)	50mL	ab138478
Guinea Pig Serum (sterile)	50mL	ab138480
Hamster Serum (sterile)	50mL	ab139500
Horse Serum (sterile)	50mL	ab139501
Mouse Serum (sterile)	50mL	ab138705
Rabbit Serum (sterile)	50mL	ab138706
Rat Serum (sterile)	50mL	ab138328
Sheep Serum (sterile)	50mL	ab138327

Non-sterile Sera

Product name	Description/size	Product code
Bovine Calf Serum	25mL	ab7479
Cat Serum	10mL	ab139724
Chicken Serum	25mL	ab7477
Dog Serum	10mL	ab139737
Donkey Serum	25mL	ab7475
Goat Serum	50mL	ab7481
Guinea Pig Serum	10mL	ab7482
Hamster Serum	10mL	ab7483
Horse Serum	25mL	ab7484
Llama Serum	20mL	ab139738
Mouse Serum	10mL	ab7486
Rabbit Serum	25mL	ab7487
Rat Serum	10mL	ab7488
Sheep Serum	50mL	ab7489

Biotin Blocking

Biotin is present in many tissues but is particularly higher in tissues such as kidney, liver or brain tissue. If an Avidin-biotin based detection system is used, blocking endogenous biotin is recommended. In-house protocols for blocking biotin are available but for a robust and reproducible protocol a biotin blocking reagent or a polymer based detection system is recommended (refer to immunostaining kits and reagents page 10).

Protocol recommendations:

1. Block endogenous biotin prior to or after incubation with primary antibody but NOT after incubation with a biotinylated secondary.
2. Wetting sample with blocking buffer and flicking this off prior to avidin-biotin blocking creates a nice wet area on and around section for avidin-biotin block

Peroxidase blocking

When using an HRP conjugated antibody for detection non-specific background staining may occur due to tissue containing endogenous peroxidase. To check for this, tissues can be incubated with DAB substrate prior to primary antibody incubation and if tissues turn brown, endogenous peroxidase is present and a blocking step is required. Hydrogen peroxide (H_2O_2) is the most commonly used peroxidase blocking agent.

Protocol recommendations:

1. Block at preferred stage of IHC protocol (i.e. after rehydration to water/before antigen retrieval, after antigen retrieval/before primary incubation, after primary incuation/before secondary incubation or after secondary antibody incubation (for certain antigens like CD4 and CD8 blocking after primary and secondary incubation is recommended as hydrogen peroxide is detrimental to epitopes).
2. Incubate section typically in 0.3% hydrogen peroxide for 10-15 minutes (incubation time may be 5-60 mins depending on concentration of hydrogen peroxide).

Alkaline Phosphatase (AP) blocking

Endogenous alkaline phophatase (AP) can produce a high background when using AP chromogen substrates. Endogenous AP can typically be found in kidney, intestine, osteoblasts, lymphoid tissue and placenta. Endogenous AP is often more prevalent in frozen tissue and blocking is recommended. Tissue can be tested for endogenous AP through incubation with BCIP/NBT, if a blue colour is observed endogenous AP is present and blocking is necessary.

Protocol recommendations:

1. Endogenous AP can be blocked by including levamisole in the chromogen substrate. Chromogens containing levisamole are typically available commercially.
2. For intestinal tissue AP blocking is recommended by treatment with a weak acid prior to application of the primary antibody.

Autofluorescence blocking

When using a fluorescent label for detection there is a possibility that the unprocessed or fixed tissue maybe autofluorescent. Test samples should be carried out to ensure that the tissue being studied is not inherently fluorescent or that fixation steps do not induce autofluorescence. Potential methods to reduce autofluorescence are outlined below:

Table 6. Autofluorescence blocking guidelines

Method 1	Using frozen tissue sections to reduce possibility of induced autofluorescence during fixation
Method 2	Reduce aldehyde presence during fixation by treating tissue with sodium borohydride or glycine/lysine
Method 3	Treating tissue with quenching dyes such as Pontamine sky blue, Sudan black or Trypan blue or FITC block

If no solution is found to autofluorescence the use of an enzymatic detection system may be preferable.

Blocking cross reactive antigens

Mouse on Mouse

When staining mouse tissues with mouse primary antibodies, high background may be observed as endogenous mouse IgG will be detected by the secondary targeting the exogenous mouse antibody. To reduce this background in-house, protocols are available from Abcam but to produce robust and reproducible staining a mouse on mouse kit is recommended.

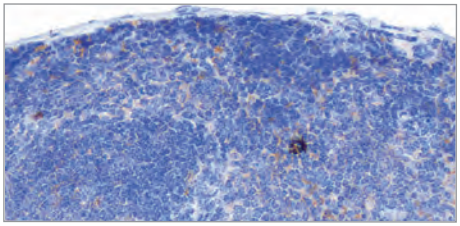
Mouse on Mouse detection kit (ab127055)

Featured Product.

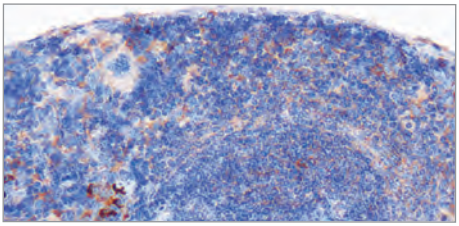
- Minimal background
- Strong staining through polymer based detection
- Biotin-free detection in biotin enriched tissue
- Simple and reliable protocol for reproducible experiments

Reduction in background staining of endogenous mouse IgG demonstrated in mouse spleen and mouse colon using the Mouse on Mouse detection kit (ab127055):

Reduced endogenous mouse IgG background

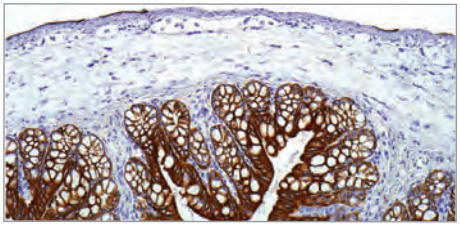


Negative control image, using Mouse on Mouse detection kit (ab127055) on mouse spleen. (Formalin/PFA-fixed paraffin-embedded sections).

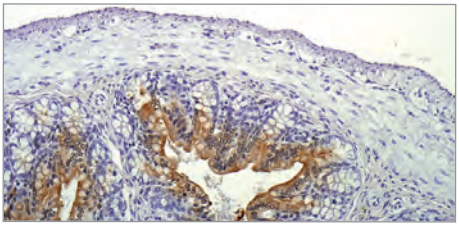


Negative control image, using EXPOSE detection kit (ab80436) on mouse spleen. (Formalin/PFA-fixed paraffin-embedded sections).

Stronger staining with Mouse on Mouse detection system



Mouse Pan CK (Clone AE1/AE3) antibody staining Pan CK in mouse colon with the use of ab127055. (Formalin/PFA-fixed paraffin-embedded sections).



Mouse Pan CK (Clone AE1/AE3) antibody staining Pan CK in mouse colon using ABC Mouse on Mouse system. (Formalin/PFA-fixed paraffin-embedded sections).

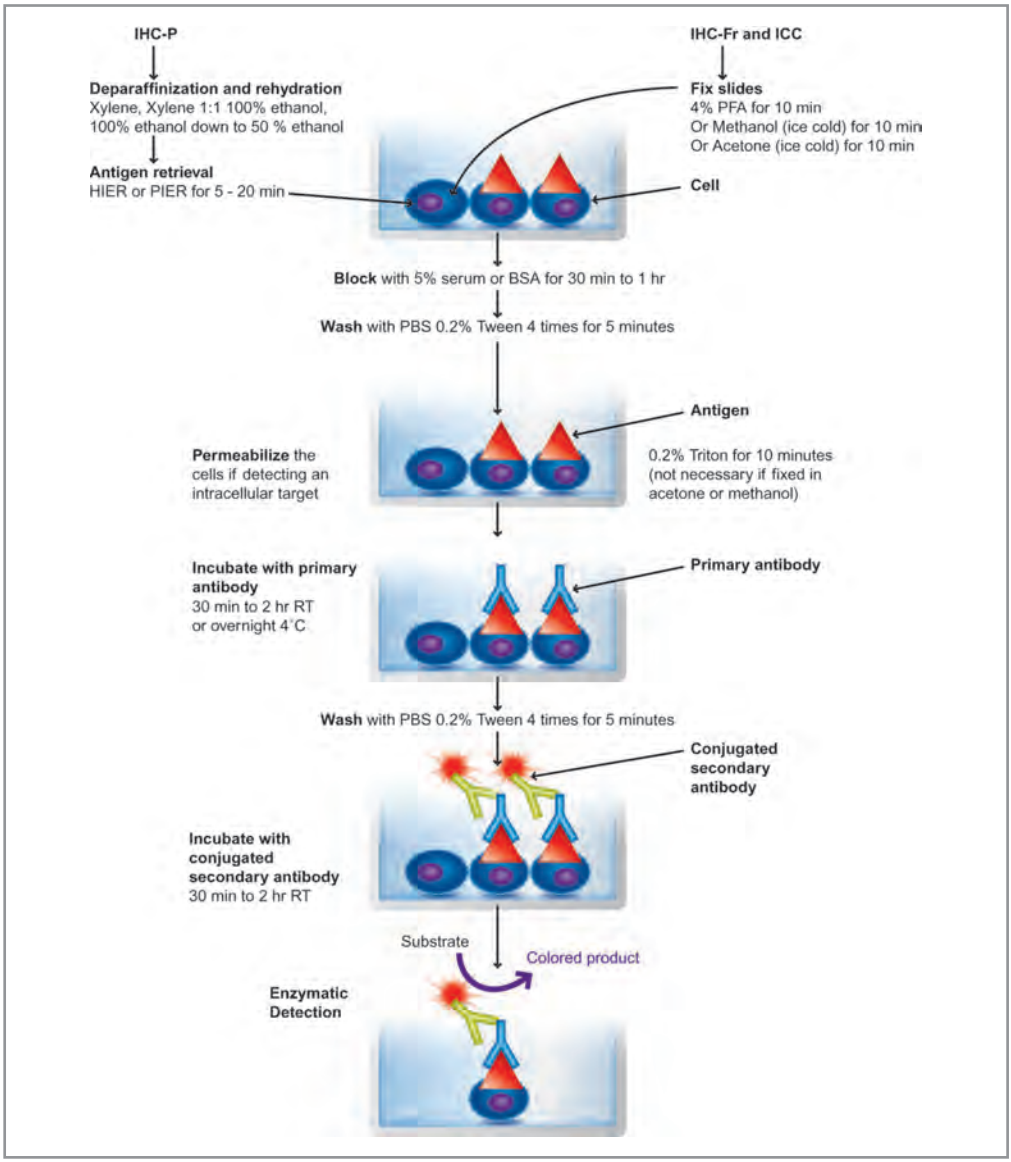
Reagents for Blocking

Product name	Size/description	Product code
Endogenous Avidin/Biotin Blocking Kit	15mL	ab64212
Endogenous Avidin + Biotin Blocking System	15mL Avidin, 15mL Biotin	ab3387
FITC Protein Blocking Agent (PBA)	6mL	ab128980
Hydrogen Peroxide Blocking Reagent	125mL	ab64218
Hydrogen Peroxide Blocking Reagent	60mL	ab94666
Protein Block	125mL	ab64226
Protein Block	60mL	ab156024

Immunostaining and Reagents for immunostaining

Immunostaining relies on the detection of specific antibody-antigen interactions with an antibody that has been tagged with a visible label, typically a fluorescent dye, colloidal metal or an enzyme. The steps in a typical chromogenic and fluorescent immunostaining protocol are shown below:

Figure 3. Immunostaining flow diagram

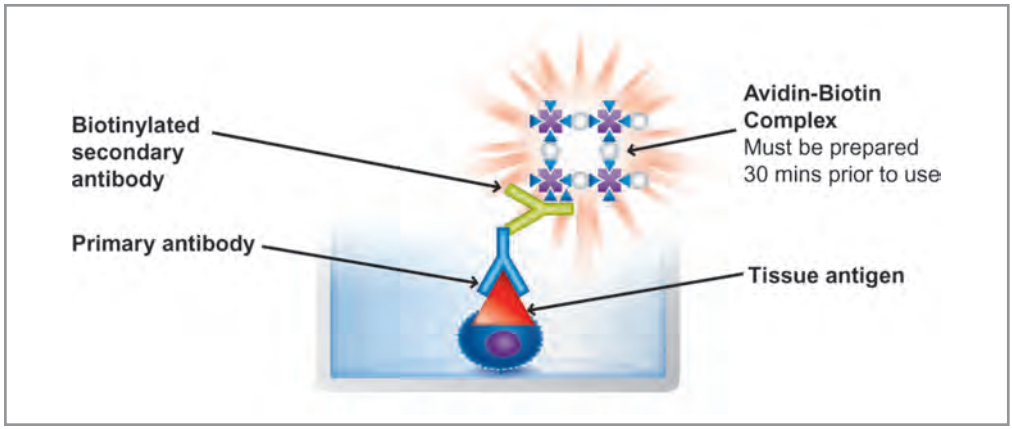


Immunostaining kits

There are a number of different labelling techniques that can be used for immunostaining that use in-direct or direct detection of the target antigen. In-direct methods include the use of an avidin-biotin complex (ABC) or a labeled streptavidin-biotin complex (LSAB). Direct methods include the use of a polymer complex or a micro-polymer complex.

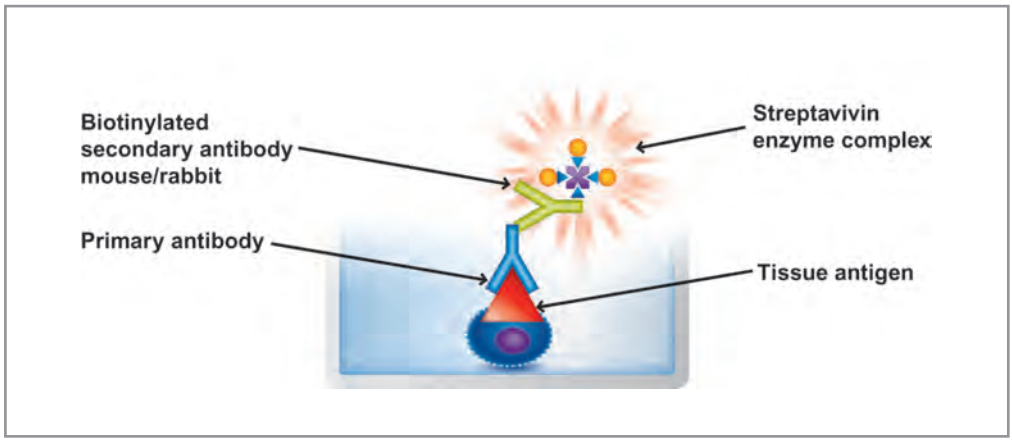
The ABC method relies on secondary antibodies that are conjugated to biotin to act as links between tissue bound primary antibodies and an avidin-biotin-peroxidase complex.

Figure 4. Avidin-Biotin Complex (ABC) method



The LSAB method is similar to the ABC method and uses a biotinylated secondary antibody that links primary antibodies to a streptavidin-peroxidase conjugate. The advantage of the LSAB method is that in comparison to avidin, streptavidin has a more neutral isoelectric point and lacks carbohydrate moieties resulting in less nonspecific tissue binding. An advantage of in-direct detection methods is that due to the large enzyme to antibody ratio there is a degree of signal amplification which provides high sensitivity.

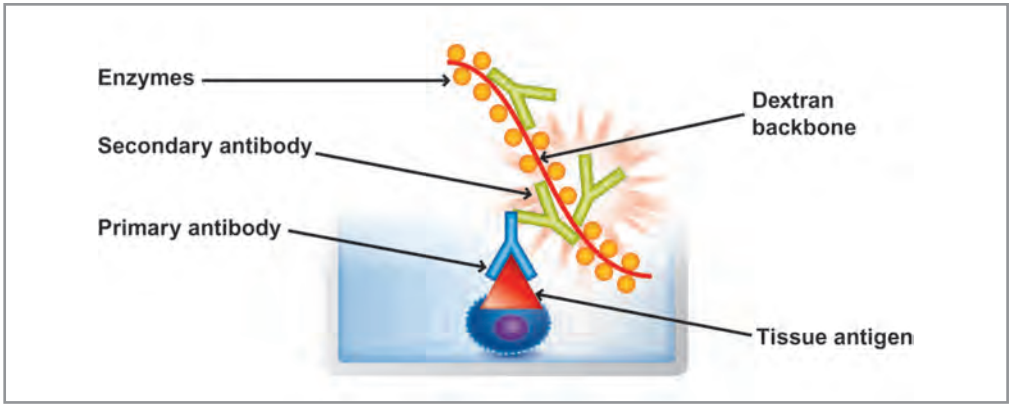
Figure 5. Labeled Streptavidin-Biotin (LSAB) method



Although streptavidin-biotin based detection systems are still widely used there are a number of limitations associated with using these methods. The key challenge with these methods is that the presence of endogenous biotin can lead to significant background staining in certain circumstances (e.g. with kidney or brain tissue), this can be worse when staining frozen sections where levels of endogenous biotin tend to be higher than in paraffin-embedded specimens. Direct based detection methods offer a solution to the challenge of endogenous biotin background as well as offering simpler protocols and comparable if not better staining to ABC methods.

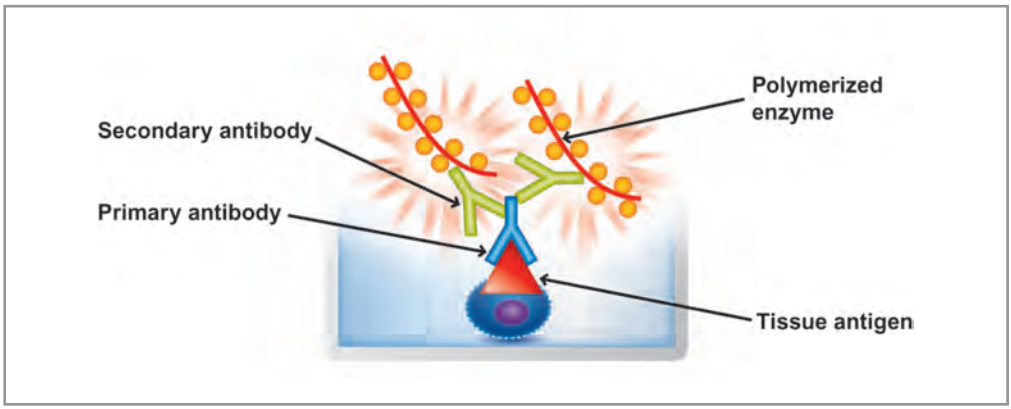
In polymer based methods a dextran backbone is utilized to which multiple enzyme molecules and secondary antibodies are attached, the dextran backbone and secondary complex then binds to the respective primary antibody.

Figure 6. Polymer method



More recently, micro-polymer (or compact polymer) based detection methods are available that eliminate the need for a dextran backbone as the enzyme is polymerized directly onto the secondary antibody which forms a smaller detection complex. The main advantages offered by the smaller detection complex are greater sensitivity through better tissue penetration and an improved signal to noise ratio with no endogenous biotin being stained.

Figure 7. Micro-polymer method



The use of ready-to-use detection kits offers increased reproducibility and consistency. You can find a range of streptavidin-biotin and micro-polymer based (EXPOSE) detection kits available from Abcam.

Biotin-Streptavidin Detection Systems

Abcam's catalogue includes a number of kits that utilise the LSAB detection system. Choose HRP Plus detection kits for enhanced sensitivity.

Mouse and Rabbit specific kits (anti-polyvalent)

Product name	Size/description	Product code
Mouse and Rabbit specific HRP/DAB (ABC) Detection IHC Kit	15mL	ab64264
Mouse and Rabbit specific HRP/DAB Plus (ABC) Detection IHC Kit	60mL	ab93697
Mouse and Rabbit specific HRP/DAB Plus (ABC) Detection IHC Kit	125mL	ab94698
Mouse and Rabbit specific HRP/AEC (ABC) Detection IHC Kit	15mL	ab93705
Mouse and Rabbit specific HRP/AEC Plus (ABC) Detection IHC Kit	125mL	ab93677
Mouse and Rabbit specific HRP AEC or DAB (ABC) Detection IHC Kit	1L	ab94669
Mouse and Rabbit specific AP (ABC) Detection IHC Kit	60mL	ab93695
Mouse and Rabbit specific AP (ABC) Detection IHC Kit	125mL	ab94725
Mouse and Rabbit specific AP/BCIP/NBT (ABC) Detection IHC Kit	15mL	ab128966
Mouse and Rabbit specific AP/Fast Red (ABC) Detection IHC Kit	15mL	ab128967

Rabbit specific kits:

Product name	Size/description	Product code
Rabbit specific HRP/DAB (ABC) Detection IHC Kit	15mL	ab64261
Rabbit specific HRP/AEC (ABC) Detection IHC Kit	15mL	ab64260
Rabbit specific HRP (ABC) Detection IHC Kit	60mL/125mL	ab128973
Rabbit specific AP/BCIP/NBT (ABC) Detection IHC Kit	15mL	ab128968
Rabbit specific AP/Fast-Red (ABC) Detection IHC Kit	15mL	ab128969
Rabbit specific AP (ABC) Detection IHC Kit	60mL/125mL	ab128972

Mouse specific kits:

Product name	Size/description	Product code
Mouse specific HRP/DAB (ABC) Detection IHC Kit	15mL	ab64259
Mouse specific HRP/AEC (ABC) Detection IHC Kit	15mL	ab64258
Mouse specific HRP (ABC) Detection IHC Kit	60mL/125mL	ab128971
Mouse specific AP/BCIP/NBT (ABC) Detection IHC Kit	15mL	ab128964
Mouse specific AP/Fast-Red (ABC) Detection IHC Kit	15mL	ab128965
Mouse specific AP (ABC) Detection IHC Kit	60mL/125mL	ab128970

Note: Abcam ABC detection IHC kits use an LSAB detection system

EXPOSE IHC (micro-polymer) Detection Sytems

Benefit from the advantages of using a micro-polymer/compact-polymer detection system. The range available from Abcam offers you greater sensitivity as well as improved signal to noise ratio.

Mouse and rabbit specific kits

Product name	Size/description	Product code
EXPOSE Mouse and Rabbit specific HRP/DAB detection IHC kit	15ml	ab80436
EXPOSE Mouse and Rabbit specific HRP/DAB detection IHC kit	60ml	ab94710
EXPOSE Mouse and Rabbit specific HRP/DAB detection IHC kit	125ml	ab94709
EXPOSE Mouse and Rabbit specific HRP/AEC detection IHC kit	15ml	ab93686
EXPOSE Mouse and Rabbit specific HRP/AEC detection IHC kit	60ml	ab94705
EXPOSE Mouse and Rabbit specific HRP/AEC detection IHC kit	125ml	ab94706
EXPOSE Mouse and Rabbit specific AP (red) detection IHC kit	15ml	ab94734
EXPOSE Mouse and Rabbit specific AP (red) detection IHC kit	60ml	ab94735
EXPOSE Mouse and Rabbit specific AP (red) detection IHC kit	125ml	ab94736
EXPOSE Mouse and Rabbit specific HRP/DAB or AEC detection IHC kit	1L	ab93702

Rabbit specific kits

Product name	Size/description	Product code
EXPOSE Rabbit specific HRP/DAB detection IHC kit	15ml	ab80437
EXPOSE Rabbit specific HRP/DAB detection IHC kit	60ml	ab94726
EXPOSE Rabbit specific HRP/DAB detection IHC kit	125ml	ab94727
EXPOSE Rabbit specific HRP/AEC detection IHC kit	15ml	ab94361
EXPOSE Rabbit specific HRP/AEC detection IHC kit	60ml	ab94728
EXPOSE Rabbit specific HRP/AEC detection IHC kit	125ml	ab94729
EXPOSE Rabbit specific AP (red) detection IHC kit	15ml	ab94737
EXPOSE Rabbit specific AP (red) detection IHC kit	60ml	ab94738
EXPOSE Rabbit specific AP (red) detection IHC kit	125ml	ab94739

Mouse specific kits

Product name	Size/description	Product code
EXPOSE Mouse specific AP (red) detection IHC kit	15ml	ab94740
EXPOSE Mouse specific AP (red) detection IHC kit	60ml	ab94743
EXPOSE Mouse specific AP (red) detection IHC kit	125ml	ab94747

Substrates and Chromogens

When using enzymatic detection chromogen/substrates are catalyzed at the site of an enzymatic label to produce a colored precipitate that can be visualised. The Chromogen to be used is dependent on the enzyme being used. The table below offers guidelines to selecting the appropriate label.

Table 7. Substrates and Chromogens for IHC

Enzyme	Chromogen/ substrate	Color	Advantages	Disadvantages	Mounting media
Horseradish Peroxidase (HRP)	AEC	**Red**	Intense color; contrasts well with blue in double staining	Endogenous peroxidase activity in tissue can lead to false positive staining	Aqueous
	DAB	**Brown**	Intense color; permanent		Organic
	DAB + Nickel enhancer	**Black**	Intense color; permanent		Organic
	TMB	**Blue**	Intense color; permanent		Aqueous
Alkaline Phosphatase (AP)	BCIP/NBT	**Blue**	Intense color	Endogenous alkaline phosphatase activity in tissue can lead to false positives. Fast Red and Fast Blue TR Prone to fading	Organic
	Naphthol AS-MX phosphate + fast blue BB	**Blue**	Less intense, good for double staining		Aqueous
	Naphthol AS-MX phosphate + fast red TR	**Red**	Less intense, good for double staining		Aqueous
	Naphthol AS-MX phosphate + new fuchsin	**Red**	Intense color		Organic
Glucose Oxidase	NBT	**Blue**	No endogenous enzyme activity	Low staining intensity. High antibody concentrations needed	Organic

Substrate and chromogen products

Product name	Size/description	Product code
AEC Single/Plus	30mL	ab103742
AEC Substrate System (Ready to Use)	125mL	ab64252
Alkaline Phosphatase chromogen (BCIP/TNBT)	100mL	ab7413
Alkaline Phosphatase chromogen (BCIP/NBT)	100mL	ab7468
Alkaline Phosphatase Enhancer	250mL	ab671
DAB Enhancer	10mL	ab675
DAB Substrate Kit	125mL	ab64238
DAB Substrate Kit	60mL	ab94665
Fast-Red Substrate System	60mL	ab128979
Permanent Fast-Red Substrate System	60mL or 125mL	ab128992
Liquid Fast-Red Substrate Kit	60mL	ab128981
Liquid Fast-Red Substrate Kit	125mL	ab64254
StayBlue/AP (Alcohol and Xylene substitute compatible)	30mL	ab156428
Stay Green/AP (Alcohol and Xylene substitute compatible)	30mL	ab103745
StayRed/AP (Alcohol and Xylene compatible)	30mL	ab103741
Steady DAB/Plus	200mL	ab103723
Streptavidin Alkaline Phosphatase (Ready to Use)	125mL	ab64268
Streptavidin Alkaline Phosphatase (Ready to Use)	60mL	ab128984
Streptavidin Peroxidase (Ready to Use)	125mL	ab64269
Streptavidin Peroxidase (Ready to Use)	60mL	ab128985

Mounting media

Mounting of a specimen is essential to preserve the specimen during immunostaining and storage in addition to enhancing image quality during microscopy.

Our standard Mounting media can be used to mount setions that have been stained with either DAB or AEC chromogens. Ultra Plus and Vision Mounting Media can be used with either DAB, AEC or FastRed chromogens

Mounting Media for IHC:

Product name	size/description	Product code
Aqueous Mounting Medium	6mL	ab128982
BrightMount	25mL	ab103746
BrigthMount/Plus	25mL	ab103748
Mounting Medium	125mL	ab64230
Vision Mounting Medium	125mL	ab94702

IHC controls

It is essential to run controls in IHC staining experiments to confirm that the observed staining pattern is true, accurate and reliable. Two types of controls are required for the tissue type:

Antigen controls:

- **Positive control:** A section from a cell line or tissue known to express the protein you are detecting. A positive result from the positive control, even if the samples are negative, will indicate the procedure is optimized and working. It will verify that any negative results are valid.
- **Negative control:** A section from a cell line or tissue sample known not to express the protein you are detecting. This is to check for non specific binding and false positive results.

Reagent control:
This ensures that staining is produced from primary antibody staining the antigen and not from detection system or the specimen. This can be determined by using the detection system with diluent alone and no primary antibody.

Tissue slides for IHC

Abcam offers a range of over 230 tissue slides in both diseased and normal state that can be used as positive staining controls in immunohistochemistry as well as in *in-situ* hybridization. Tissue types included in the range include spleen, kidney, angioma and Alzheimer tissues.

Further information can be found online at **www.abcam.com/tissueslides**

IHC counterstains and special stains

After immunostaining of a tissue a second stain is often used to provide contrast that is valuable in making the primary antibody stain stand out. Counterstains are available for chromogenic or fluorescent detection. Popular counterstains are outlined below:

Counterstaining products

Common counterstains and their targets.

Type	Dye	Target	Color	Product code
Chromogenic	Hematoxylin	Nuclei	Blue to violet	ab1288990
Chromogenic	Nuclear fast red (Kernechtrot)	Nucleic acids	Red	ab128992
Chromogenic	Methyl green	Nucleic acids	Green	-
Fluorescent	DRAQ7™	Nucleic acids	Red	-
Fluorescent	Nuclear yellow	Nucleic acids	Yellow	ab138903
Fluorescent	Nuclear Green DCS1	Nucleic acids	Green	ab138905
Fluorescent	Hoechst stain	Nucleic acids	Blue	-
Fluorescent	4', 6-diamidino-2-phenylindole (DAPI)	Nucleic acids	Blue	-
Fluorescent	Propidium iodide	Nucleic acids	Red	ab14083
Fluorescent	Fluorophore-tagged phalloidin	Filamentous actin	Fluorophore-specific	-

Special stains

Immunohistochemistry staining can also utilise special staining kits for staining specific cellular or tissue morphology or structures using light microscopy or fluorescence.

Hydroxystilbamidine (ab138870)

Featured Product:

Hydroxystilbamidine (ab138870) also known as Fluoro-Gold™ is a fluorescent dye that can be injected *in vivo* with flexible post injection survival times. Hydroxystilbamidine (ab138870) can be used as a retrograde enhancer to label neurons.

Secondary antibodies for IHC

If using an indirect detection protocol then selecting a secondary antibody is necessary, if not provided with the detection system. Secondary antibodies also provide signal amplification compared to direct detection as more than one secondary antibody will bind to the primary.

The secondary antibody should be directed against the species the primary antibody was raised in (i.e. if a primary raised in rabbit has been used an anti-rabbit secondary antibody raised in a species other than rabbit must be used). It is also important that the isotype of your secondary antibody matches your primary antibody. Generally, affinity purified antibodies are the most popular as they provide the lowest amount of non-specific binding. However IgG fractions can also potentially contain very high affinity antibodies and may be useful when an antigen is poorly expressed or in low abundance.

Pre-adsorbed secondary antibodies are useful for reducing non-specific background as they are less likely to show species cross-reactivity or to react with endogenous antigens of the species they have been pre-adsorbed against.

The secondary antibody should therefore, be pre-adsorbed against the same species the sample originated from. For example, it is advisable to use a secondary antibody pre-adsorbed against human serum when staining human tissues or cell lines. For more information, please go to www.abcam.com/pre-adsorption.

$F(ab')_2$ fragment secondary antibodies are recommended for staining of tissues rich in Fc receptors (eg. spleen, thymus, blood etc..) to eliminate non specific binding. $F(ab')_2$ fragment secondary antibodies as they are smaller and therefore more easily penetrate tissues, are particularily useful for multiple IHC staining.

Secondary antibodies can be either enzyme labeled (peroxidase, alkaline phophatase), fluorochrome labeled (FITC, R-PE, Alex-Fluor®) or biotinylated.

Abcams catalogue contains a range of biotinylated secondary antibodies for use in ABC (avidin biotin complex) detection sytems.

Optimized IHC secondary antibodies

Product name	Description/size	Product code
Biotinylated Goat anti Mouse IgG (H+L) (Ready-To-Use)	125mL	ab64255
Biotinylated Goat anti Mouse IgG (H+L) (Ready-To-Use)	60mL	ab128976
Biotinylated Goat anti Rabbit IgG (H+L) (Ready-To-Use)	125mL	ab64256
Biotinylated Goat anti Rabbit IgG (H+L) (Ready-To-Use)	60mL	ab128978
Biotinylated Goat anti Mouse & Rabbit IgG (H+L) (Ready-To-Use)	125mL	ab64257
Biotinylated Goat anti Mouse & Rabbit IgG (H+L) (Ready-To-Use)	60mL	ab128977
Goat anti Mouse IgG secondary antibody (H+L), pre-absorbed	1mg	ab64244
Goat polyclonal to Peroxidase anti-Peroxidase complex / PAP antibody	1mL	ab28054
Mouse polyclonal to Peroxidase anti-Peroxidase complex / PAP antibody	100µl	ab21867

Discover other secondary antibodies at **www.abcam.com/secondaries**

Using directly labeled primary antibodies for IHC

An alternative to using a secondary antibody for detection in IHC is to use a directly labeled primary antibody. Directly labeled antibodies are suitable for well expressed antigens, for more poorly expressed antigens a secondary detection step is recommended in order to benefit from amplification from the secondary reagent.

Using direct detection the primary antibody can be conjugated to an enzyme such as horse radish peroxidase (HRP) or alkaline phosphatase (AP) or alternatively to a fluorochrome. The benefit of direct detection is an additional incubation step with a secondary reagent is not necessary. An additional and significant benefit of direct detection is that when using fluorochromes for direct detection, is increased flexibility in the design of multiple staining experiments with the wide range of fluorochromes that are available.

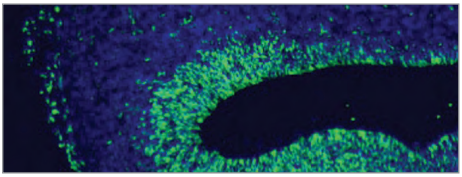
EasyLink

Featured Product:

To benefit from direct detection, discover a wide choice of over 25 fluorescent and enzymatic labels for direct conjugation to your primary antibody in the EasyLink range.

Recommended primary antibodies for IHC

Selected cancer markers



Featured Product:

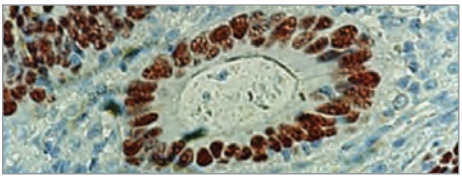
Antibody description:
Mouse monoclonal [PC10] to PCNA - Proliferation Marker (ab29)

Species reactivity:
Chk, Dfsh, Dm, Hu, Mk, Ms, Rat, Zfsh

Applications:
Flow Cyt, ICC/IF, IHC-Fr, IHC-P, IP, WB

PCNA is an auxiliary protein of DNA polymerase delta and is involved in the control of eukaryotic DNA replication by increasing the polymerase's processibility during elongation of the leading strand. Induces a robust stimulatory effect on the 3'-5' exonuclease and 3'-phosphodiesterase, but not apurinic-apyrimidinic (AP) endonuclease, APEX2 activities. Has to be loaded onto DNA in order to be able to stimulate APEX2.

The image shows ab29 staining of PCNA in mouse embryonic brain tissue section by Immunohistochemistry (Frozen sections).



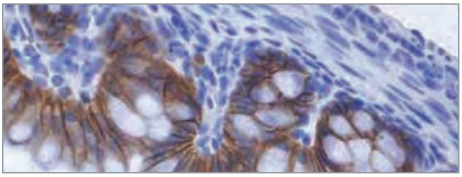
Featured Product:

Antibody description:
Mouse monoclonal [PAb 240] to p53 (ab26)

Species reactivity:
Cow, Dog, Hu, Ms, Rat, SHm

Applications:
Flow Cyt, ICC/IF, IHC-Fr, IHC-P, IP, WB

P53 acts as a tumor suppressor in many tumor types; induces growth arrest or apoptosis depending on the physiological circumstances and cell type. Involved in cell cycle regulation as a trans-activator that acts to negatively regulate cell division by controlling a set of genes required for this process. One of the activated genes is an inhibitor of cyclin-dependent kinases. Apoptosis induction seems to be mediated either by stimulation of BAX and FAS antigen expression, or by repression of Bcl-2 expression. Implicated in Notch signaling cross-over. Isoform 2 enhances the transactivation activity of isoform 1 from some but not all TP53-inducible promoters. Isoform 4 suppresses transactivation activity and impairs growth suppression mediated by isoform 1. Isoform 7 inhibits isoform 1-mediated apoptosis.
The image shows Mouse bone tissue sections stained with ab29 by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections).



Featured Product:

Antibody description:
Rabbit polyclonal to E Cadherin (ab53033)

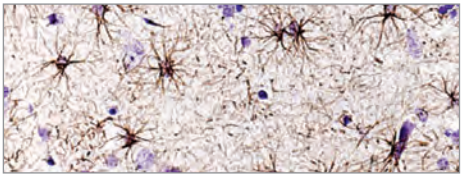
Species reactivity:
Hu, Ms, Rat, AGMk, Cow, Dog, Zfsh

Applications:
ELISA, Flow Cyt, ICC/IF, IHC-Fr, IHC-P, IP, WB

Cadherins are calcium-dependent cell adhesion proteins. They preferentially interact with themselves in a homophilic manner in connecting cells; cadherins may thus contribute to the sorting of heterogeneous cell types. CDH1 is involved in mechanisms regulating cell-cell adhesions, mobility and proliferation of epithelial cells. Has a potent invasive suppressor role. It is a ligand for integrin alpha-E/beta-7.
E-Cad/CTF2 promotes non-amyloidogenic degradation of Abeta precursors. Has a strong inhibitory effect on APP C99 and C83 production.
The image shows ab53033 staining of E Cadherin in mouse colon tissue section by Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections).

Product description	Species reactivity	Applications	Product code
Rabbit polyclonal to Aurora B	Hu, Ms, Rat, Hm	ICC/IF, IHC-Fr, IHC-P, IP, WB	ab2254
Rabbit monoclonal [Y69] to c-Myc	Hu, Ms, Rat	ICC/IF, IHC-P, IP, WB	ab32072
Rabbit polyclonal to CD31	Hu, Ms	ICC/IF, IHC-FoFr, IHC-Fr, IHC-FrFl, IHC-P, WB	ab28364
Rabbit monoclonal [EP774Y] to EGFR (phospho Y1092)	Hu, Ms	ICC/IF, IHC-FoFr, IHC-P, WB	ab40815
Mouse monoclonal [IST-9] to Fibronectin	Hu, Ms, Rat, Chk, Cow, Dog, Mk, Pig	ELISA, ICC/IF, IHC-Fr, IHC-P, RIA, WB	ab6328
Rabbit polyclonal to IGF2	Cow, Hu, Ms, Rat	ELISA, ICC/IF, IHC-P, Neut, sELISA, WB	ab9574
Rabbit polyclonal to N Cadherin	Hu, Ms, Rat, Chk, Cow, Zfsh	ICC, ICC/IF, IHC-Fr, IHC-P, WB	ab12221
Mouse monoclonal [CH-19] to pan Cadherin	Hu, Ms, Rat, Cat, Chk, Cow, Dog, Goat, Gpig, Hm, Pig, Rb, SRat, Snk, Xl, Zfsh	Flow Cyt, ICC, ICC/IF, IHC-Fr, IHC-P, WB	ab6528

Selected neuroscience markers



Featured Product:

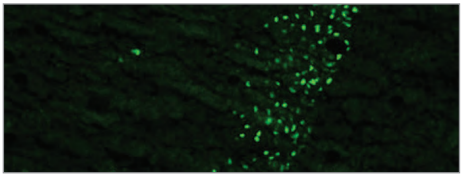
Antibody description:
Rabbit polyclonal to GFAP - Astrocyte Marker (ab7260)

Species reactivity:
Hu, Ms, Rat, Cat, Mmst

Applications:
ICC, ICC/IF, IHC-FoFr, IHC-Fr, IHC-P, WB

GFAP, a class-III intermediate filament, is a cell-specific marker that, during the development of the central nervous system, distinguishes astrocytes from other glial cells.

The image shows immunohistochemistical detection of GFAP antibody - Astrocyte Marker (ab7260) on formaldehyde-fixed paraffin-embedded monkey brain sections.



Featured Product:

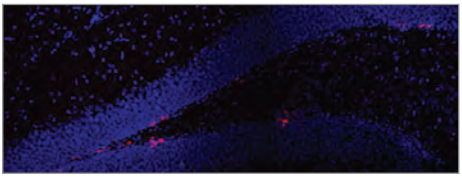
Antibody description:
Rabbit polyclonal to TBR1 (ab31940)

Species reactivity:
Hu, Ms, Rat

Applications:
ICC, ICC/IF, IHC-FoFr, IHC-Fr, IHC-P, WB

T box brain 1 (TBR1) is a member of a conserved protein family that share a common DNA-binding domain, the T-box. T-box genes encode transcription factors involved in the regulation of developmental processes. Disruption of the mouse TBR1 homolog demonstrated a critical role for TBR1 in early cortical development. TBR1 expression is largely restricted to the cerebral cortex, where during embryogenesis it distinguishes domains that give rise to the paleocortex, limbic cortex, and neocortex.
The image shows ab31940 staining of rat brain sections by IHC-Fr. The animal was perfused with 4% paraformaldehyde and further post fixed with 4% paraformaldehyde overnight.

Selected neuroscience markers *(Continued)*



Featured Product:

Antibody description:
Rabbit polyclonal to TBR2 / Eomes - ChIP Grade (ab23345)

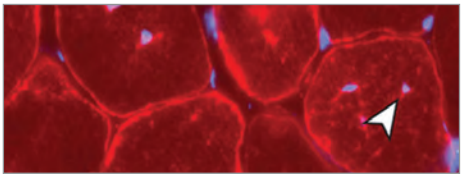
Species reactivity:
Hu, Ms, Rat, Mmst

Applications:
ChIP, ChIP/Chip, ICC/IF, IHC-FoFr, IHC-Fr, IHC-FrFl, IHC-P, WB

TBR2 functions as a transcriptional activator playing a crucial role during development. Functions in trophoblast differentiation and later in gastrulation, regulating both mesoderm delamination and endoderm specification. Plays a role in brain development being required for the specification and the proliferation of the intermediate progenitor cells and their progeny in the cerebral cortex. Also involved in the differentiation of CD8+ T-cells during immune response regulating the expression of lytic effector genes.The image shows immunohistochemistical detection of GFAP antibody - Astrocyte Marker (ab7260) on formaldehyde-fixed paraffin-embedded monkey brain sections. IHC-FoFr image of TBR2 staining in 8 week old mouse hippocampus using ab23345.

Product description	Species reactivity	Applications	Product code
Mouse monoclonal [LB 509] to alpha Synuclein	Hu, Rat	ELISA, Flow Cyt, IHC-FoFr, IHC-Fr, IHC-P, WB	ab27766
Rabbit polyclonal to Axin 2	Hu, Ms, Rat	Flow Cyt, ICC/IF, IHC-Fr, IHC-P, WB	ab32197
Rabbit monoclonal [E247] to beta Catenin	Hu, Ms, Rat, AGMk, Hm, Mcq	ICC/IF, IHC-Fr, IHC-P, IP, WB	ab32572
Rabbit polyclonal to Doublecortin - Neuronal Marker ab18723	Hu, Ms, Rat, Cat, Chk, Ql, RMk	ICC, ICC/IF, IHC-FoFr, IHC-Fr, IHC-FrFl, IHC-P, WB	
Rabbit polyclonal to Dystrophin	Dog, Hu, Ms	ICC/IF, IHC-Fr, IHC-P, WB	ab15277
Goat polyclonal to Iba1	Hu, Ms, Rat, Gpig, Pig	ICC, ICC/IF, IHC-FoFr, IHC-Fr, IHC-FrFl, IHC-P, WB	ab5076
Rabbit monoclonal [SP6] to Ki67 - Proliferation Marker	Hu, Ms, Rat	ICC/IF, IHC-FoFr, IHC-Fr, IHC-P, WB	ab16667
Rabbit polyclonal to LAMP1	Hu, Ms, Rat, Dog, Hm, Xl, Zfsh	ICC, ICC/IF, IHC-Fr, IHC-P, IP, WB	ab24170
Chicken polyclonal to MAP2 - Neuronal Marker	Hu, Ms, Rat, Cow, CynMk	ELISA, ICC/IF, IHC (PFA fixed), IHC-FoFr, IHC-Fr, IHC-P, WB	ab5392
Rat monoclonal [12] to Myelin Basic Protein - Oligodendrocyte Marker	Hu, Ms, Rat, A lept, Cow, Dog ,Gpig, Pig, Rb, Shp	ELISA, IHC-FoFr, IHC-Fr, IHC-P, RIA, WB	ab7349
Rabbit polyclonal to Parvalbumin	Hu, Ms, Rat, Chk	ELISA, ICC/IF, IHC-FoFr, IHC-Fr, IHC-P, IP, WB	ab11427
Mouse monoclonal [13C4 / I3C4] to PGP9.5 - Neuronal Marker	Hu, Ms, Rat, Dog, Gpig, Pig, Rb, Shp, Zfsh	ICC, ICC/IF, IHC-FoFr, IHC-Fr, IHC-P, WB	ab8189
Mouse monoclonal [SY38] to Synaptophysin	Hu, Ms, Rat, Cow, Hm	Flow Cyt, ICC/IF, IHC-Fr, IHC-P, WB	ab8049
Rabbit monoclonal [EPR3776] to Vimentin	Hu, Ms, Rat, RMk	Flow Cyt, ICC, ICC/IF, IHC-P, IP, WB	ab92547

Selected cardiology markers



Featured Product:

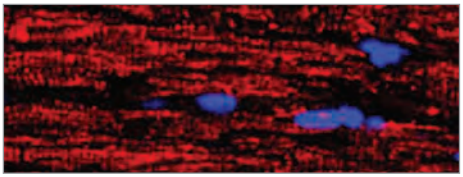
Antibody description:
Rabbit monoclonal [Y266] to Desmin (ab32362)

Species reactivity:
Hu, Ms, Rat, Gpig

Applications:
Flow Cyt, ICC/IF, IHC - Wmt, IHC-Fr, IHC-P, WB

Desmin are class-III intermediate filaments found in muscle cells. In adult striated muscle they form a fibrous network connecting myofibrils to each other and to the plasma membrane from the periphery of the Z-line structures.

The image shows immunohistochemical analysis (frozen sections) of mouse skeletal muscle tissue following cardiotoxin injury, staining Desmin with ab32362.



Featured Product:

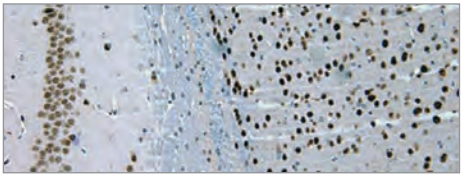
Antibody description:
Mouse monoclonal [3-48] to heavy chain cardiac Myosin (ab15)

Species reactivity:
Hu, Ms, Rat, Cow, Dog, Pig, Rb

Applications:
ELISA, Flow Cyt, ICC, ICC/IF, IHC (PFA fixed), IHC-Fr, IHC-P, WB

Cardiac myosin heavy chain (MHC) exists as two isoforms in humans, alpha-cardiac MHC and beta-cardiac MHC. These two isoforms are expressed in different amounts in the human heart. During normal physiology, beta-cardiac MHC is the predominant form, with the alpha-isoform contributing around only 7% of the total MHC. Mutations of the MHC genes are associated with several different dilated and hypertrophic cardiomyopathies.

The image shows immunohistochemical staining of paraffin embedded rat heart tissue sections by ab15.



Featured Product:

Antibody description:
Rabbit polyclonal to MEF2C (ab64644)

Species reactivity:
Hu, Ms, Rat, Mk

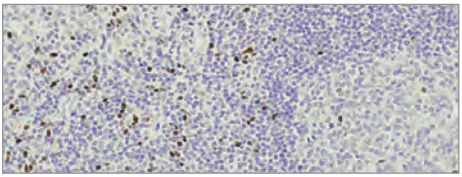
Applications:
ICC/IF, IHC-FoFr, IHC-P, WB

MEF2C is a transcription activator which binds specifically to the MEF2 element present in the regulatory regions of many muscle-specific genes. It controls cardiac morphogenesis and myogenesis, and is also involved in vascular development. It plays an essential role in hippocampal-dependent learning and memory by suppressing the number of excitatory synapses and thus regulating basal and evoked synaptic transmission. Crucial for normal neuronal development, distribution, and electrical activity in the neocortex, it is also necessary for proper development of megakaryocytes and platelets and for bone marrow B lymphopoiesis. Other functions include B-cell survival and proliferation in response to BCR stimulation, efficient IgG1 antibody responses to T-cell-dependent antigens and for normal induction of germinal center B cells. It may also be involved in neurogenesis and in the development of cortical architecture (by similarity). Isoform 3 and isoform 4, which lack the repressor domain, are more active than isoform 1 and isoform 2..

The image shows immunohistochemical staining of MEF2C in formalin-fixed, paraffin-embedded mouse brain sections with ab64644.

Product description	Species reactivity	Applications	Product code
Rabbit polyclonal to alpha smooth muscle Actin	Hu, Ms, Rat, Chk, Cow, Dog, Gpig, Pig	ELISA, ICC, ICC/IF, IHC-FoFr, IHC-Fr, IHC-P, WB	ab5694
Rabbit monoclonal [EP798Y] to Calponin	Hu, Ms, Rat, Dog, Pig	ICC/IF, IHC-Fr, IHC-P, WB	ab46794
Rabbit polyclonal to SM22 alpha	Hu, Ms, Rat, Chk, Cow, Pig	ICC, ICC/IF, IHC-Fr, IHC-P, WB	ab14106
Rabbit polyclonal to CTGF	Hu, Rat, Pig	ICC/IF, IHC-Fr, IHC-P	ab5097
Rabbit polyclonal to KAT13D / CLOCK - ChIP Grade	Hu, Ms, Rat, Hm	ChIP, GSA, ICC/IF, IHC-Fr, IHC-P, WB	ab3517
Rabbit polyclonal to Myeloperoxidase	Hu, Ms, Rat, Mk	IHC-FoFr, IHC-Fr, IHC-P	ab9535
Mouse monoclonal [HM.11] to Nitro tyrosine	-	ELISA, IHC-Fr, IHC-P, WB	ab7048
Rabbit polyclonal to Nkx2.5	Hu, Ms	IHC-Fr, IHC-P, WB	ab35842
Rat monoclonal [E13 161-7] to Sca1 / Ly6A/E	Ms	ICC/IF, IHC-P	ab51317
Mouse monoclonal [1B8] to SM22 alpha	Hu, Ms, Cow, Pig, Rb	Flow Cyt, ICC/IF, IHC-Fr, IHC-P, WB	ab28811
Mouse monoclonal [A6.1] to Thrombospondin	Hu, Ms, Rat, Cow, Dog, Hrs, Pig, Shp	Flow Cyt, ICC/IF, IHC-Fr, IHC-P, IP, WB	ab1823

Selected immunology markers



Featured Product:

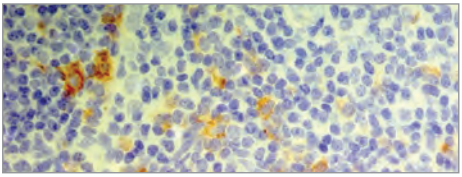
Antibody description:
Rabbit monoclonal [EP1347Y] to CD11c (ab52632)

Species reactivity:
Hu

Applications:
ICC, IHC-Fr, IHC-P, IP, WB

Integrin alpha-X/beta-2 is a receptor for fibrinogen. It recognizes the sequence G-P-R in fibrinogen. It mediates cell-cell interaction during inflammatory responses and is especially important in monocyte adhesion and chemotaxis.

The image shows immunohistochemical staining of human tonsil tissue using ab52632 (formalin/PFA-fixed paraffin-embedded sections).



Featured Product:

Antibody description:
Mouse monoclonal [236A/E7] to FOXP3 (ab20034)

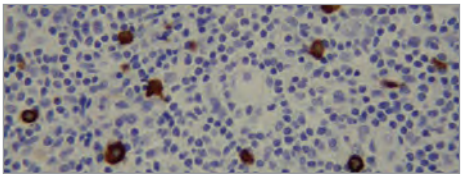
Species reactivity:
Hu, CynMk, RMk

Applications:
Flow Cyt, ICC/IF, IHC-Fr, IHC-P, WB

FOXP3 is a transcription factor that plays a critical role in the control of immune response. Defects in FOXP3 are the cause of immunodeficiency polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) [MIM:304790]; also known as X-linked autoimmunity-immunodeficiency syndrome. IPEX is characterized by neonatal onset insulin-dependent diabetes mellitus, infections, secretory diarrhea, trombocytopenia, anemia and eczema. It is usually lethal in infancy.

The image shows immunohistochemical staining of human tonsil tissue using ab20034.

Selected immunology markers *(Continued)*



Antibody description:
Mouse monoclonal [AA1] to Mast Cell Tryptase (ab2378)

Species reactivity:
Hu, Rat

Applications:
ELISA, ICC, ICC/IF, IHC-Fr, IHC-P, IHC-R, WB

Featured Product:

Mast cells contain a number of preformed chemical mediators such as histamine, chymase, carboxypeptidase and proteolytic tryptase. Human Mast Cell Tryptase is considered to be an important marker of mast cell activation as well as an important mediator of inflammation.
The image shows immunohistochemical staining of human tonsil tissue using ab2378.

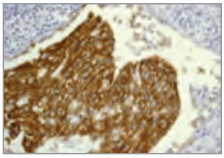
Product description	Species reactivity	Applications	Product code
Rabbit monoclonal [SP7] to CD3	Hu, Mk, Ms, Rat	IHC-FoFr, IHC-Fr, IHC-P, WB	ab16669
Mouse monoclonal [QBEND-10] to CD34	Hu, CynMk, RMk	Flow Cyt, IHC-Fr, IHC-P, IP, WB	ab8536
Mouse monoclonal [F10-44-2] to CD44	Hu	Flow Cyt, ICC/IF, IHC-Fr, IHC-P, IP	ab6124
Mouse monoclonal [KP1] to CD68	Hu, Ms	ICC/IF, IHC-FoFr, IHC-Fr, IHC-P	ab955
Rabbit polyclonal to CD274	Hu, Ms, Rat	IHC-P, WB	ab58810
Mouse monoclonal [EMR8-5] to HLA Class 1 ABC	Hu	Flow Cyt, ICC/IF, IHC-P, WB	ab70328
Mouse monoclonal [NAT] to PD1	Hu	Flow Cyt, ICC/IF, IHC-Fr, IHC-P, IP, WB	ab52587
Mouse monoclonal [TIA-1] to TIA1	Hu, Rat, Mk	IHC-P	ab2712

Discover the Rabbit Monoclonal advantage

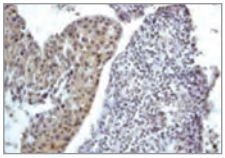
Rabbit monoclonal antibodies (RabMAbs®) combine the superior antigen recognition of a rabbit antibody with the specificity and consistency of a monoclonal. The rabbit immune system generates antibody diversity and optimizes the affinity by mechanisms that are more efficient than those of mice and other rodents. This increases the possibility of obtaining a functional antibody that will work in a variety of applications.

The rabbit monoclonal advantages:

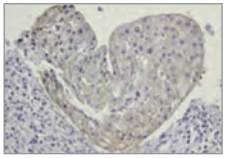
1. Low background
2. Ideal for post-translational modification detection
3. Excellent for IHC usage
4. High affinity
5. High specificity
6. Diverse/Novel epitope recognition
7. Fully validated in multiple applications
8. Ideal for use on mouse samples



Her RabMAb - 3ng / mL



Rabbit polyclonal antibody (Vendor A) - 20ng / mL



Mouse monoclonal (Vendor B) - 30ng / mL

Featured Product:

Comparison of a Her2 RabMAb with leading commercially available Her2 rabbit polyclonal (vendor A) and mouse monoclonal (vendor B) antibodies on FFPE human breast carcinoma tissue.

IHC Worksheet

(Photocopy this worksheet to help planning your experiments)

Sample No.	Tissue / cell (type, species, disease state, format)	Fixation (buffer, concentration, temperature, duration)

Type of antigen retrieval (if required)	Antigen retrieval (buffer, pH, composition, duration, temperature)	Blocking step(s) (composition, duration, temperature)

IHC Worksheet

(Photocopy this worksheet to help planning your experiments)

Sample No.	Primary antibody (diluent, concentration, duration, temperature)	Detection system (type, concentration, duration, temperature, label, chromogen)

Mounting media	Additional notes

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