Optimization Tips and Application Guide for Immunohistochemistry (IHC) and Immunofluorescent (IF)

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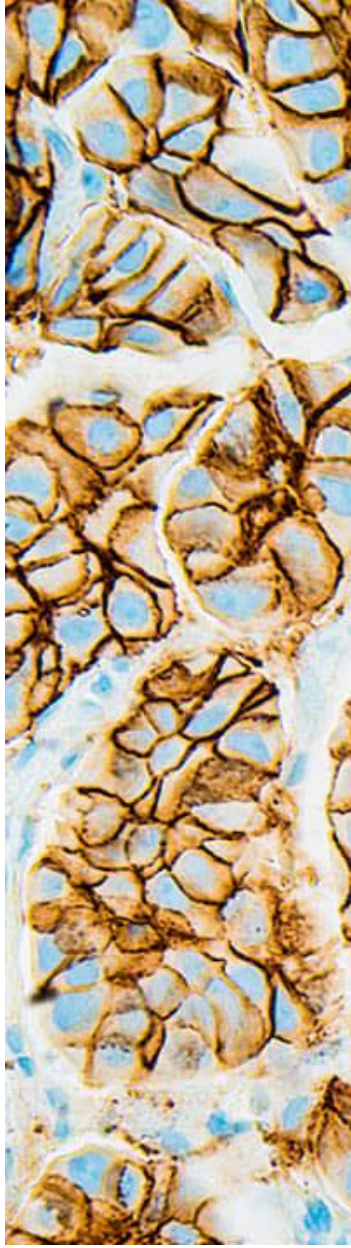
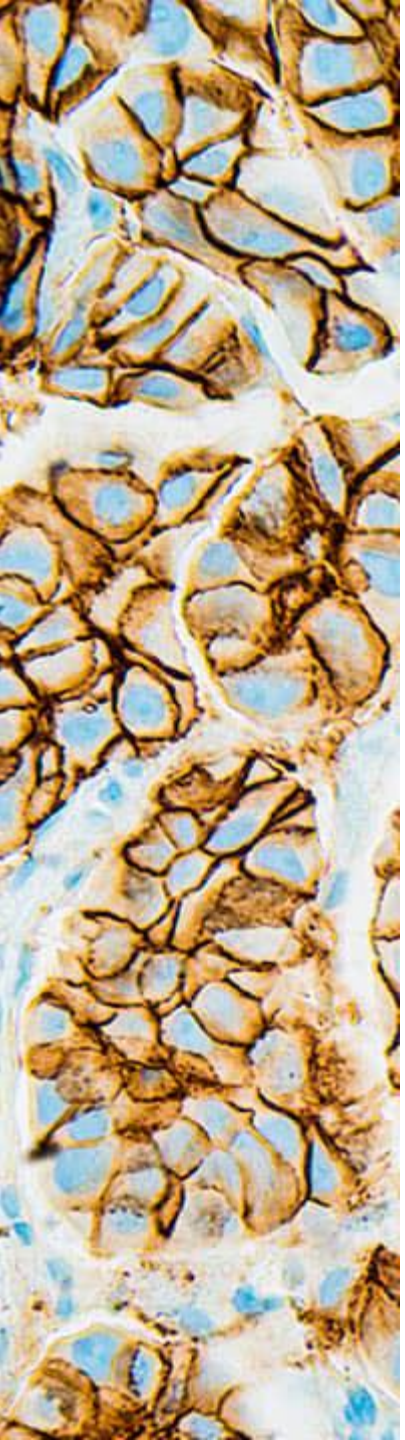


abcam

BLOSSOM



OVERVIEW

- BRIEF of IHC and IF
- Tissue staining (IHC) workflow
- ICC/IF staining workflow
- Troubleshooting
- Summary
- Q&A

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Immunohistochemistry (IHC) V.S. Immunofluorescence (IF)

Abbreviation	Assay priciple	Sample	Detection method
IHC	Immuno	histo	chemistry
	Immunology	**Tissue**	**Chemical Reaction**
ICC	Immuno	cyto	chemistry
	Immunology	**Cell**	**Chemical Reaction**
IF	Immuno		fluorescence
	Immunology	Tissue/cell	Fluorescence Dye
IHF	Immuno	histo	fluorescence
	Immunology	Tissue	Fluorescence Dye

Tissue staining (IHC) staining

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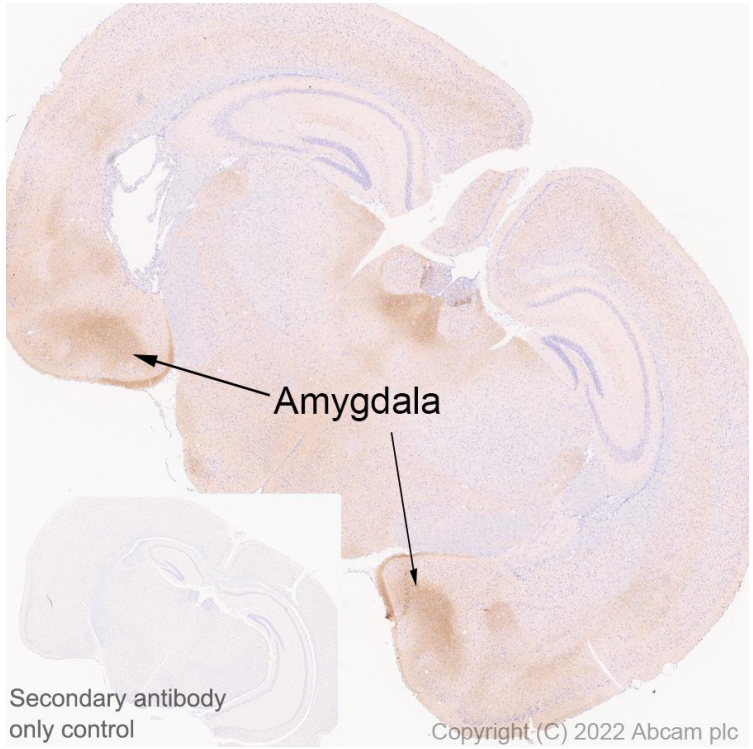
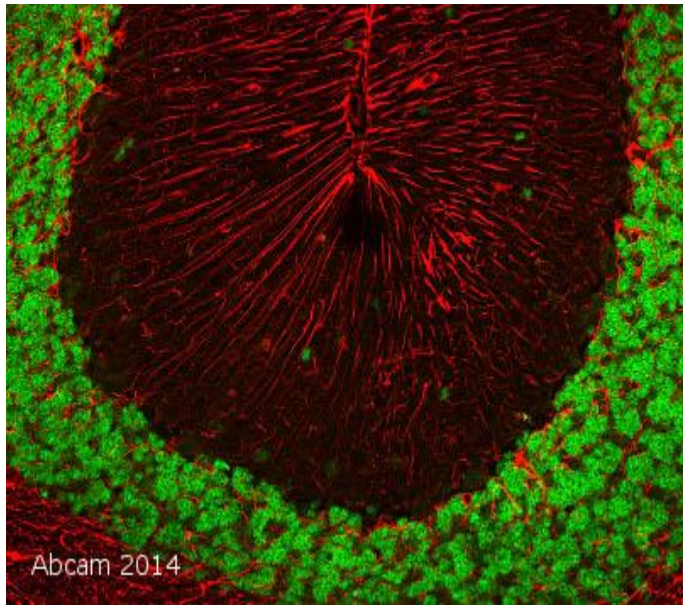
Function of Immunohistochemistry (IHC)

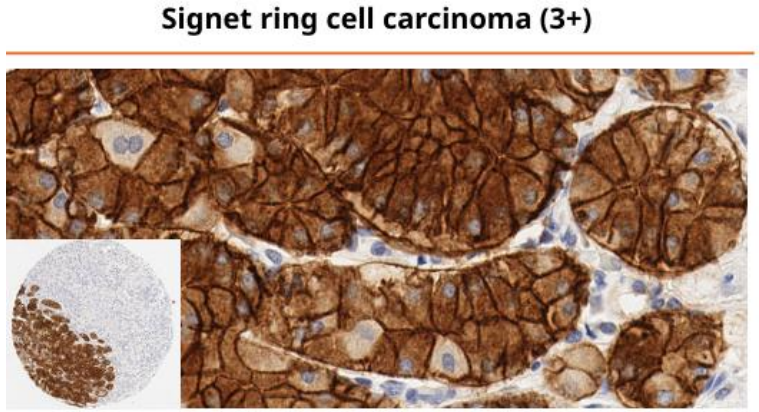


ab305032
anti-GRIN3A antibody

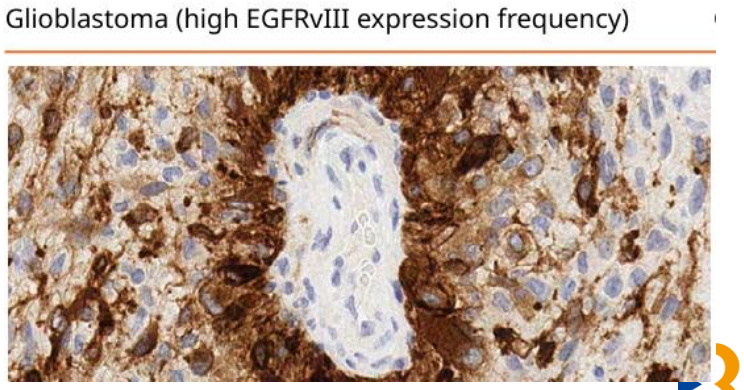


ab177487 anti-NeuN
ab4674 anti-GFAP

Ab222512
Anti-Claudin 18.2 antibody



ab313646
Anti-EGFRvIII antibody [EPR28380-83]



Location, quantity, morphology, status, diagnostic, prognosis……

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IHC Workflow

Sample preparation

1. Fixation
2. Embedding
3. Sectioning

Immunostaining protocol

4. Deparaffinization
5. Antigen retrieval
6. Blocking
7. Detection systems
8. Signal amplification
9. Counterstains & Mounting

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Fixation

Why do we need fixation?

– For tissue preservation and stabilization in its *in vivo* (life-like) state.

– To prevent autolysis and bacterial attack of tissues.

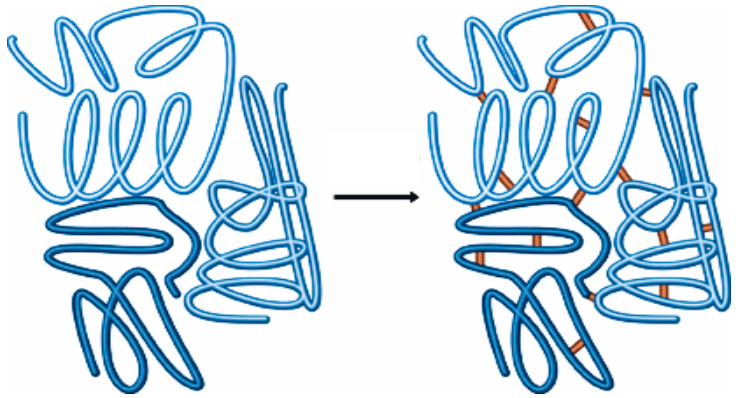
– To present antigens in tissues.

– To enhance the refractive index of tissue constituents and give tissue support during sectioning.

Without fixation, you will lose your protein (target antigen) during the tissue processing stage and see no staining!

Fixatives:

Aldehydes (cross-linking)



'methylene bridges'

- **Paraformaldehyde:** powder of polymerized formaldehyde (Can not fix tissues.)
- **Formaldehyde:** 4% solution = 10% formalin.
- **Formalin:** formaldehyde solution (37% by weight, 40% by volume) + 10–15% methanol.
- **Glutaldehyde:** 2.5%
- **Glyoxal:** 3% pH4-5

Tips:

–18-48 hours, 10-50 times larger than the volume of the tissue.
– Formalin preserves cellular structures and tissue morphologies well.
– **Antigen retrieval needed**.

Fixatives:

Alcoholic Fixatives (precipitants, coagulants)

- **Alcohols:** ice-cold (-20°C) methanol or ethanol. Disruption of internal hydrophobic bonds.
- **Acetone:** ice-cold (-20°C) acetone, generally mild.

Tips:

– **No antigen retrieval needed!**
– Methanol and Acetone will also permeabilize, no permeabilization step required.
– Protein coagulation and tissue shrinkage
– Ideal for **Frozen section**、antigens sensitive to aldehyde fixation and some PTM proteins.

Fixatives:

Aldehyde Fixatives (cross-linking):
Formaldehyde, Glutaraldehyde

Alcoholic Fixatives (precipitants, coagulants):
Acetone, Ethanol, Methanol

Mercuric chloride-based fixatives: (cross-linking):
B-5 and Zenker's

Frozen

Effect of fixation on immunostaining patterns and results

1. Volume: 10-50X of tissue
2. Size of tissue: around 5 mm thick (not larger than 2 cm)
3. Time: 18-48 hrs (around 1mm/hr)
4. Temperature: RT (for phosphoprotein: 4°C + RT)

Fix Samples AS SOON AS POSSIBLE!

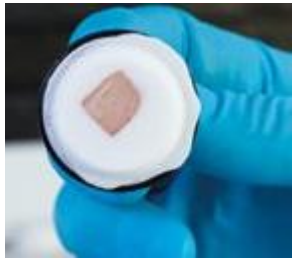
Embedding

Why do we need to embed tissues?

1. Preserving tissue morphology and giving the tissue support during sectioning.

2. Maintaining the tissue orientation is critical for preparing sections suitable for diagnosis and study objectives.

Specimen Formats For IHC Staining



	IHC-P	IHC-Fr
Embed compound	Paraffin wax	OCT
Fixation	Prior to embedding (PFA, Formalin)	Pre/post-sectioning (PFA or Alcohols)
Sectioning	Microtome, usually 4 μm	Cryostat, 5-10 μm
Storage	Long-term, multiple years (RT, 4°C or -20°C)	Short-term, 1 year (-80°C, longer at -190°C)
Advantages	Preserves tissue morphology	Preserves enzyme & antigen function (ex: PTM)
Limitations	Over fixation can mask the epitope (antigen retrieval)	Ice crystal formation may negatively affect tissue structure (cryopreservation)

Embedding

1. With paraffin wax
   - Fixed Tissue
   - Dehydration in alcohol
   - Clearing in xylene
   - Paraffin infiltration (~58-60°C)
   - Embedding

2. With OCT
   - Freeze tissues by dry-ice cooled isopropanol
   - Sucrose could be used for cryopreservation

Make sure the orientation before embedding

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Sectioning

- The cutting blade should be clean and sharp.
- Adhesive / charged glass slides are recommended (gelatin, poly-lysin…).
- Dry the sections before storage.
- Freshly sectioning the slide before staining is better.

Baking the slides before staining

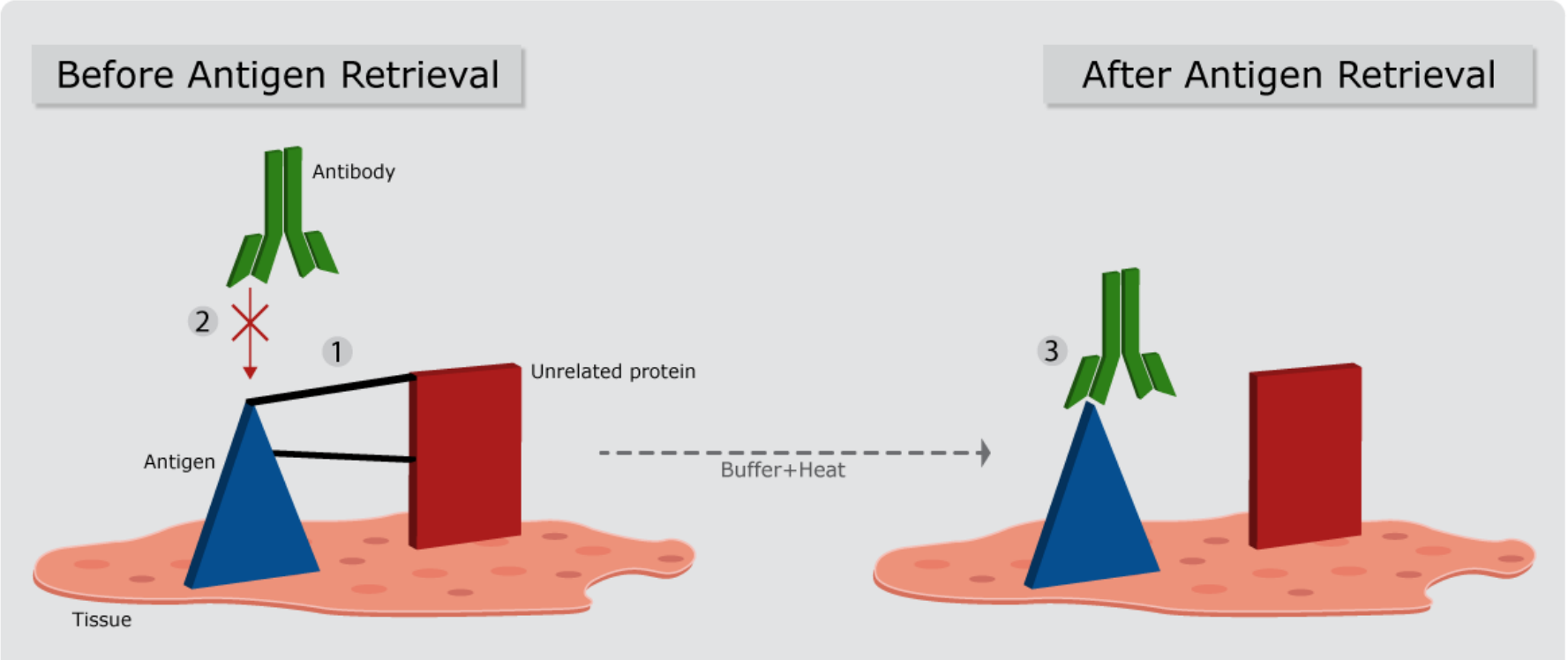
1. Bake the slides before staining to ensure the sections adhere.

2. Bake the slides at 55-60°C for 30-60 min in the oven.

3. For lipid-rich tissues (ex. Breast tissue), baking for extended time or increasing temperature.

Antigen retrieval

→ recovering the epitopes for antibodies detection



Heat-induced (HIER) VS. Proteolytic-induced (PIER)

	HIER	PIER
Advantages	Gentler epitope retrieval	Useful for epitopes that are difficult to retrieve
Buffer composition	Sodium citrate, Tris-EDTA pH dependent	Typically, pH 7.4 [Pepsin, proteinase K, trypsin]
Temperature	Approximately 95°C	Typically, 37°C
Incubation time	10–20 minutes	10–15 minutes
Precautions	Uneven and tissue dissociation	May damage the morphology

Blocking

→ Preventing Non-Specific Signals

1. **Protein blocking**
2. **Endogenous biotin**
3. **Endogenous enzymes**
4. **Autofluorescence**

1. Protein blocking:

– Blocking with serum (eg 10% normal serum): eliminates Fc receptor binding.

– Blocking with BSA (eg 1% BSA): eliminates protein-protein interactions (eg hydrophobic interactions)

2. Blocking endogenous biotin

– For avidin/biotin-based detection system

– By pre-incubation of the tissue with avidin, followed by incubation with biotin to block additional biotin binding sites on the avidin molecule.

– High in kidney, liver and brain.

3. Blocking endogenous enzymes

– **Peroxidase** blocking: [ex: Kidney, liver, or vascular areas with red blood cells]
When using **HRP (horseradish peroxidase)**-conjugated antibody for detection
Common blocking agent: **0.3-3% Hydrogen peroxide (H2O2)**

Substrate: DAB, AEC…

–**Phosphatase** blocking: [ex: Intestine, kidney, lymphoid]
When using an **AP (Alkaline phosphatase)**-conjugated antibody for detection
Common blocking agent: 1 mM **levamisole**, **weak acid** (1% acetic acid)

Substrate: Fast red, BCIP/NBT…

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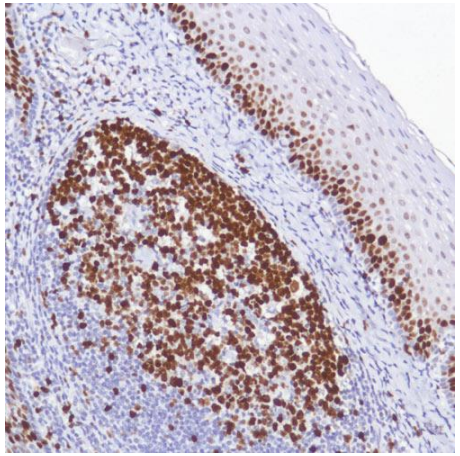

Immunohistochemical analysis of Human Tonsil tissue labeling Ki-67 anti-Mouse and Rabbit specific HRP (ABC) Detection IHC kit.

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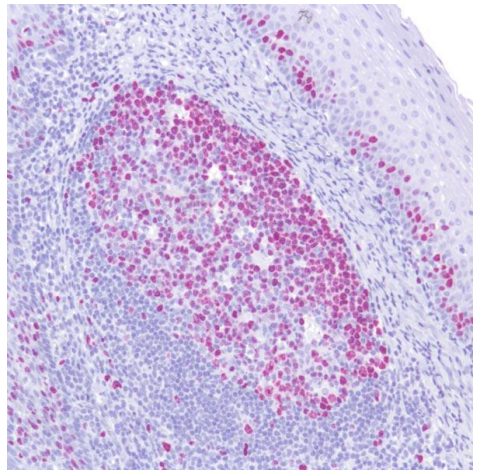

Immunohistochemical staining of human tonsil with Anti-Ki67 antibody [SP6] (ab16667).

4. Autofluorescence

Types of Autofluorescence

- **Natural autofluorescence:**

Ex: pancreas, brain, red blood cells, other pigmented cell types, lipofuscin, extracellular matrix components.

- **Induced autofluorescence:**

Aldehyde fixatives (Glutaaldehyde, Formaldehyde)

–Frozen sections are not exposed to formaldehyde for long enough to increase autofluorescence.

4. Autofluorescence

Types of Autofluorescence

- **Natural autofluorescence:**

Ex: pancreas, brain, red blood cells, other pigmented cell types, lipofuscin, extracellular matrix components.

- **Induced autofluorescence:**

Aldehyde fixatives (Glutaraldehyde, Formaldehyde)

–Frozen sections are not exposed to formaldehyde for long enough to increase autofluorescence.

Confirm autofluorescence before staining.

Autofluorescence Blocking Guidelines

- **Quenching dyes for Natural autofluorescence** [ex: sky blue, Sudan black or trypan blue etc]

— **Lipofuscin**-like autofluorescence in the brain can be blocked by treating the sections with 0.1-1% Sudan Black in 70% alcohol for 5-20 min.

— **Elastin & collagen** fibers in blood vessels: incubate sections in 0.5% sky blue for 10min.

Autofluorescence Blocking Guidelines

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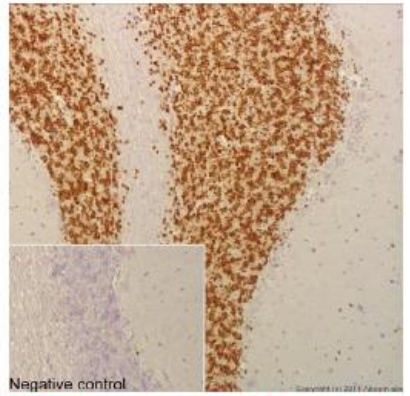
- **Glycine blocking** (Aldehyde fixatives)
- **Use frozen tissue instead** (if the autofluorescence comes from fixatives)
- **Far red dyes** (eg Alexa Fluor® 647) may give a better signal/noise ratio.

Detection systems

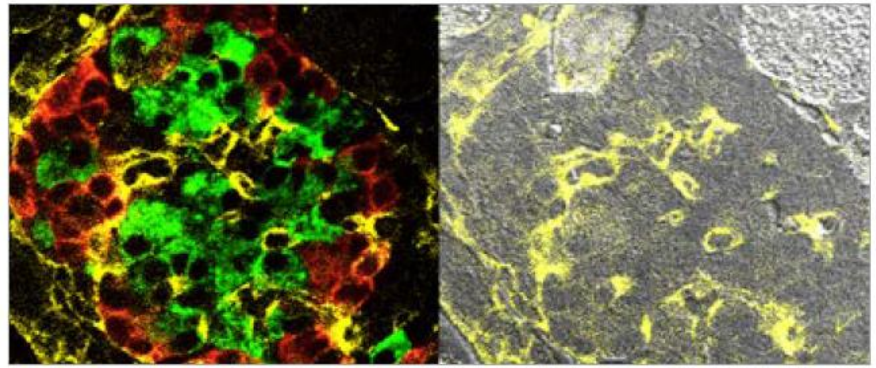
Chromogenic or Fluorescent Detection Method?

- The characteristics of your tissues (biotin, autofluorescence…)?
- Do you need to label more than two targets?
- Do you need to identify any co-localized targets?
- Do you need to preserve samples for an extended time?



Recombinant RabMAb

Anti-NeuN antibody

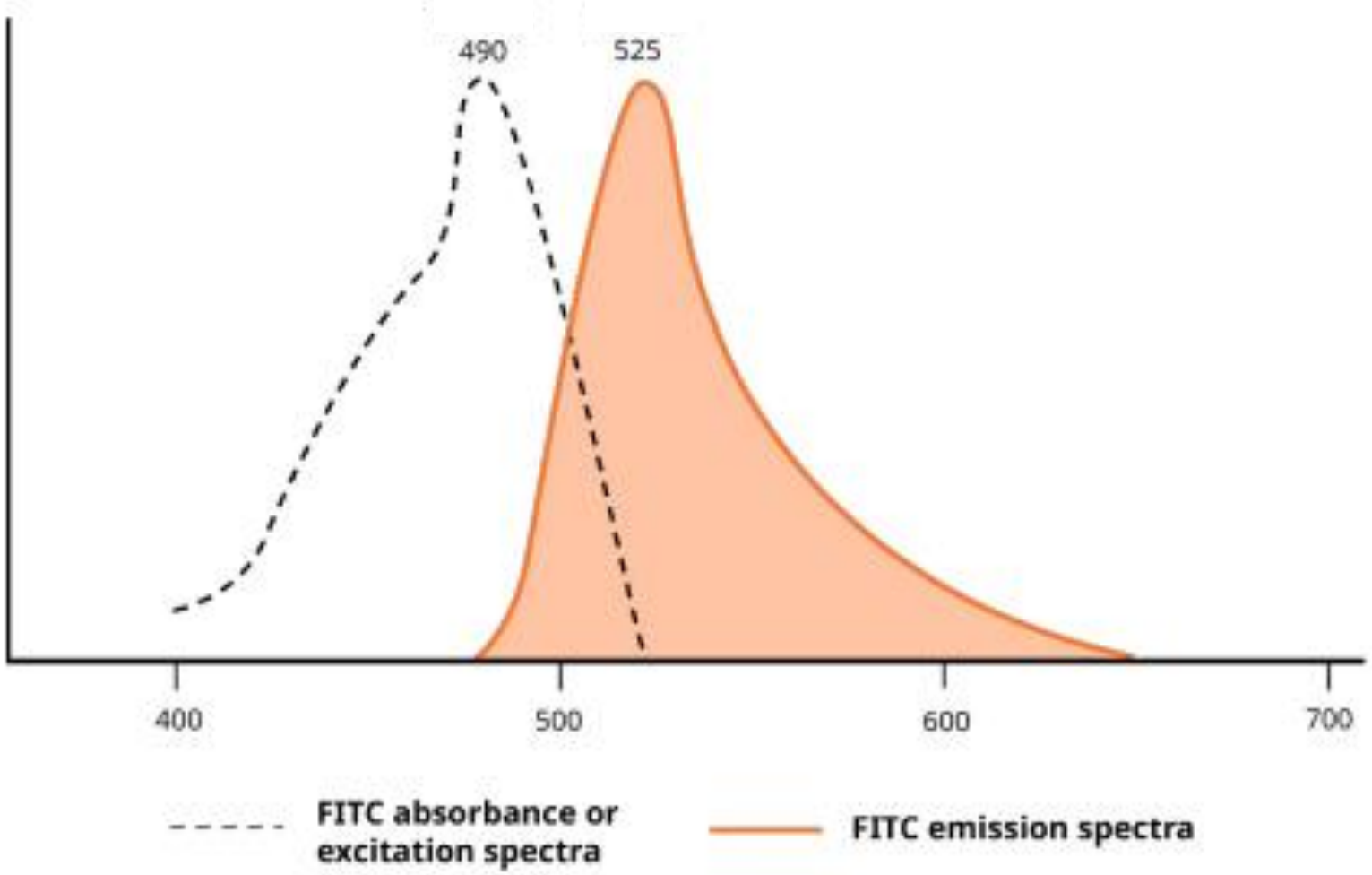


Multi-color fluorescent IHC staining of neonatal pancreas in mice using collagen IV (yellow), insulin (green), and glucagon (red) primary antibodies, and Cy2, Cy5 and Texas Red-conjugated secondary antibodies. Image from Miller K et al. PLoS One 4(11): e7739

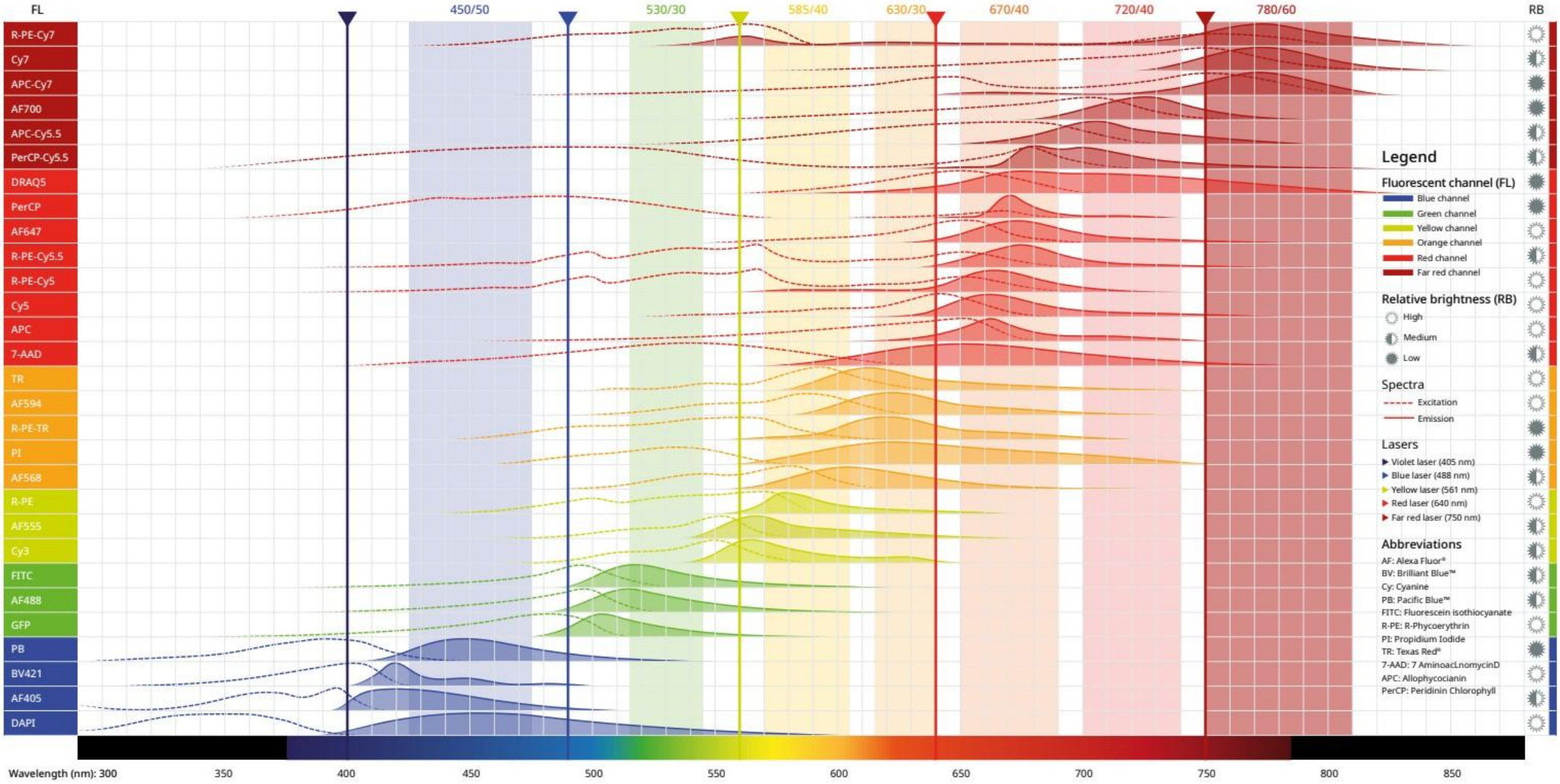
Characteristics of common chromogenic reporters used for IHC

Enzyme label	Chromogens	Precipitate Color	Recommended Nuclear Counterstain
HRP	AEC	Red	Hematoxylin
	DAB	Brown	Hematoxylin
	TMB	Blue/Dark Blue	Neutral Red / Methyl green
AP	BCIP/NBT	Dark Blue	Neutral Red
	Fast Red	Red	Hematoxylin

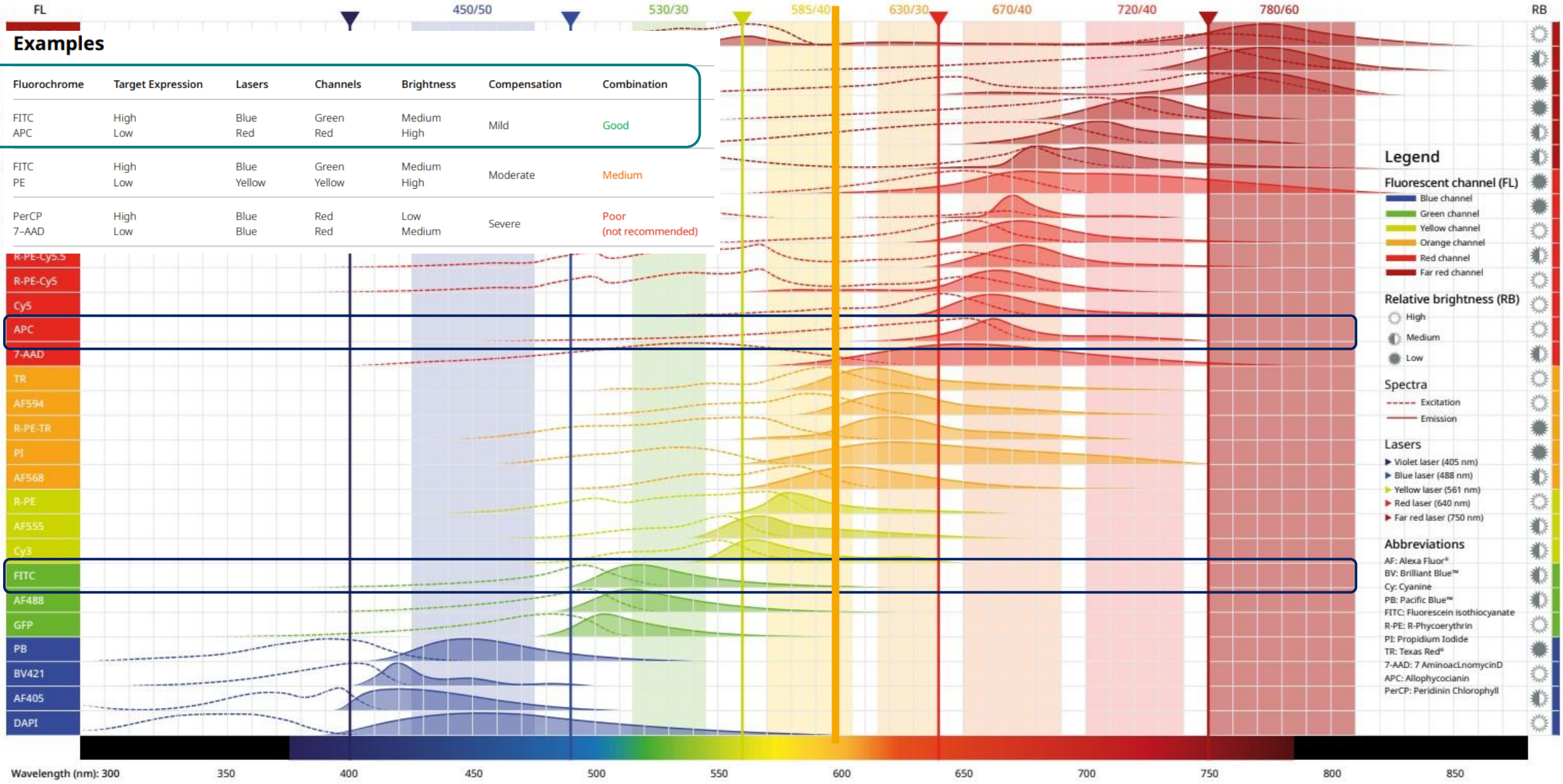
Fluorescence dye selection



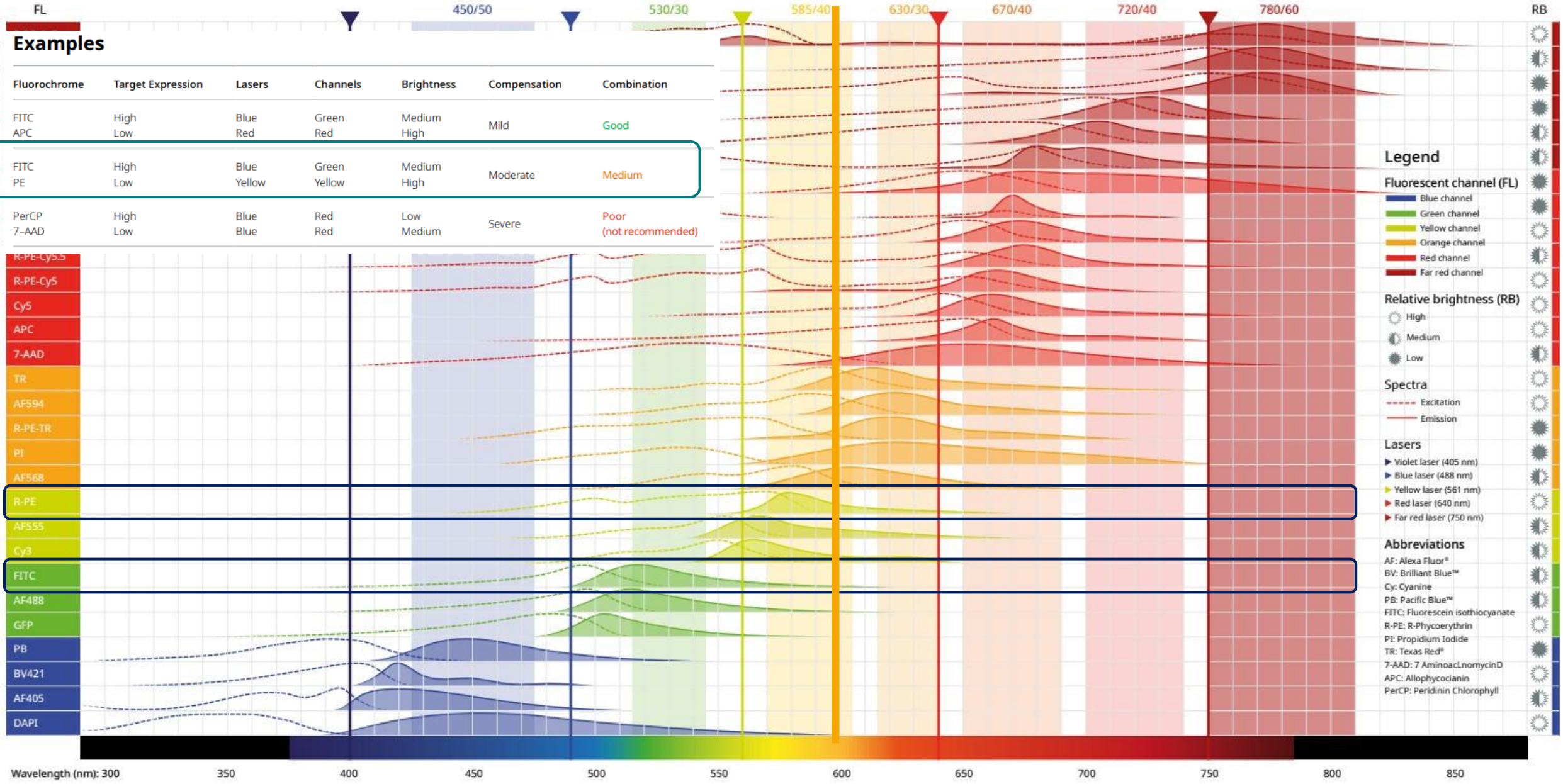
Fluorescence dye selection



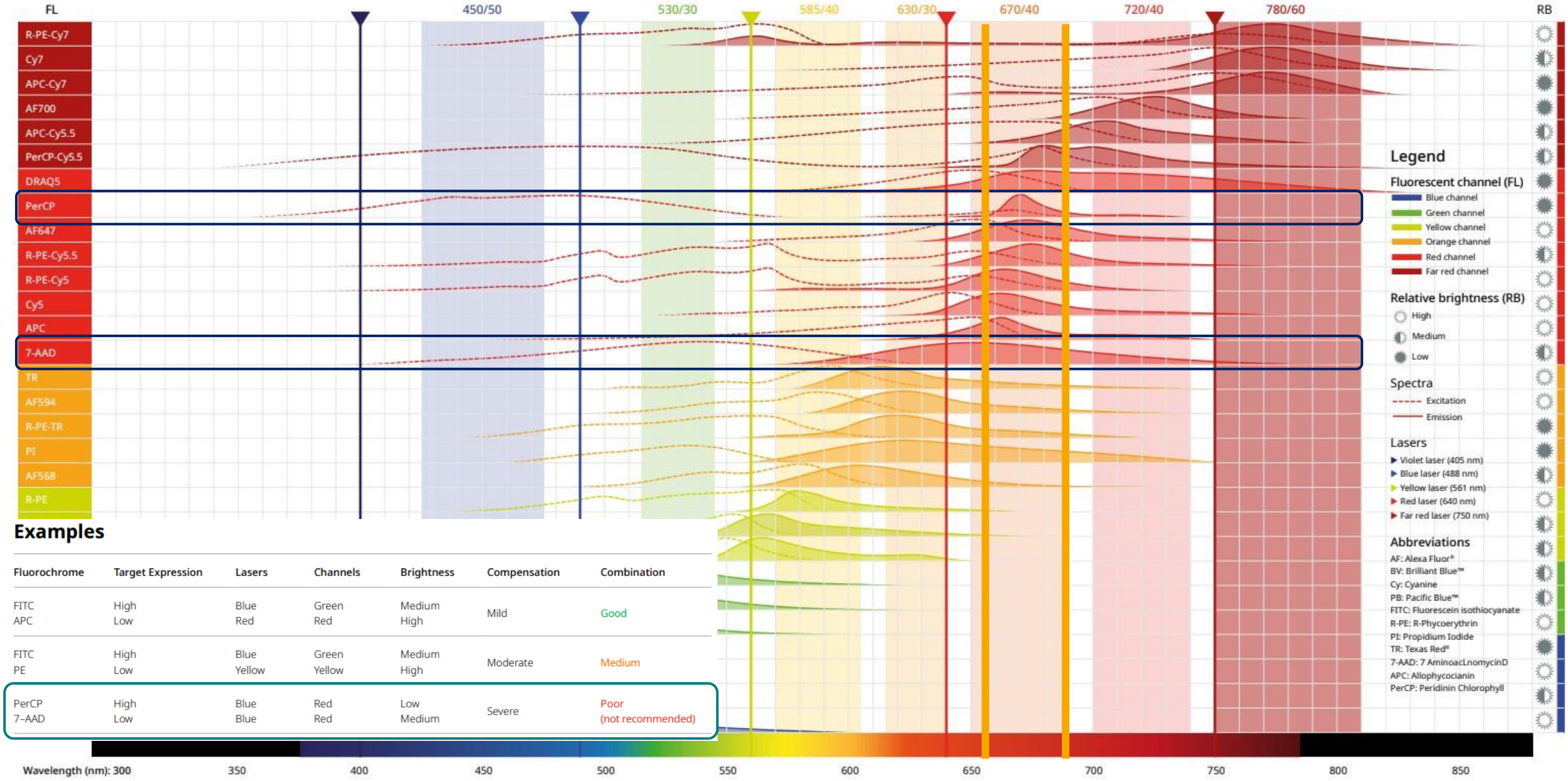
Fluorescence dye selection



Fluorescence dye selection



Fluorescence dye selection

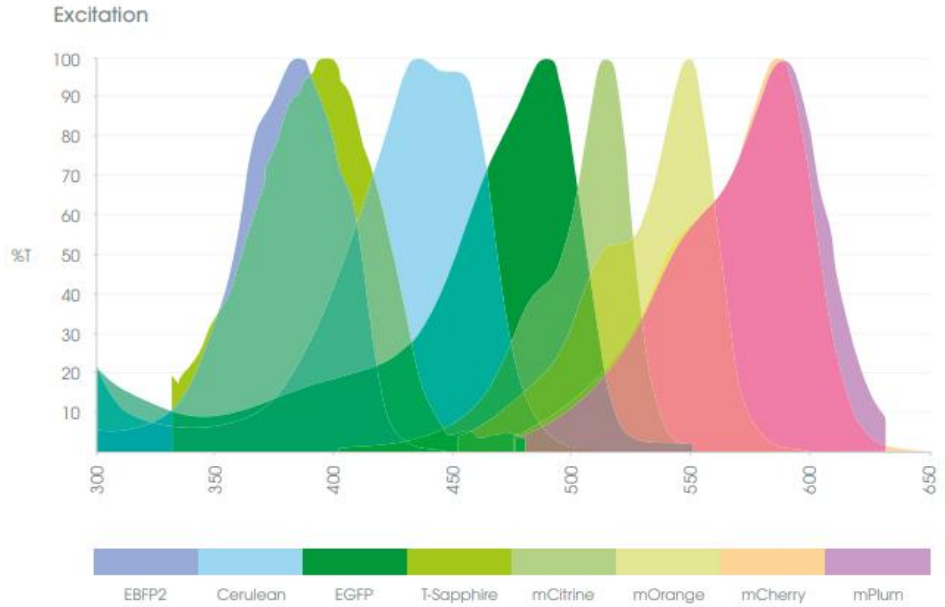


Examples

Fluorochrome	Target Expression	Lasers	Channels	Brightness	Compensation	Combination
FITC APC	High Low	Blue Red	Green Red	Medium High	Mild	Good
FITC PE	High Low	Blue Yellow	Green Yellow	Medium High	Moderate	Medium
PerCP 7-AAD	High Low	Blue Blue	Red Red	Low Medium	Severe	Poor (not recommended)

Fluorescence dye selection

Excitation and emission spectra

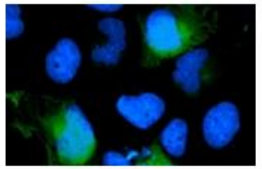


Fluorescent protein characteristics

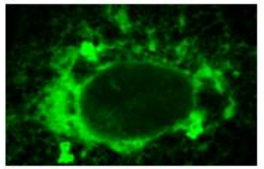
Class	Protein	λ_{ex} (nm)	λ_{em} (nm)	Extinction coefficient	Quantum Yield	Relative brightness (% of EGFP)	pKa	Bleaching t½ (s)
Blue	EBFP2	383	448	32,000	0.56	53	4.5	55
Cyan	Cerulean	433	475	43,000	0.62	79	4.7	36
Green	EGFP	488	507	56,000	0.60	100	6.0	174
UV-excitable green	T-Sapphire	399	511	44,000	0.60	79	4.9	25
Yellow	mCitrine	516	529	77,000	0.76	174	5.6	49
Orange	mOrange	548	562	71,000	0.69	146	6.5	9
Red	mCherry	587	610	72,000	0.22	47	<4.5	96
Far red	mPlum	590	649	41,000	0.10	12	<4.5	53

Antibodies

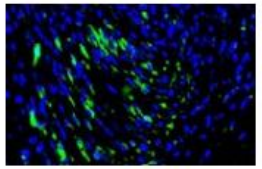
Enhance your fluorescent protein signal



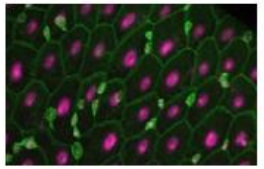
Anti-GFP antibody – ChIP Grade (ab290)
Human HEK 293 cells transfected with CACNA1D-GFP



Anti-GFP antibody (EPR14104) (ab183734)
BHK cells were transfected with GFP-Sec61 beta.



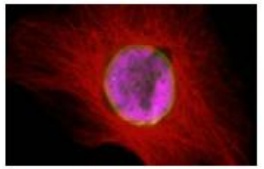
Anti-mCherry antibody (1C51) (ab125096)
Rat smooth muscle cells in bladder.



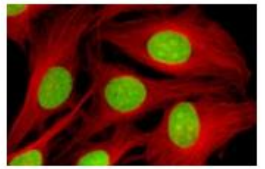
Anti-GFP antibody (ab13970)
Drosophila melanogaster tissue: adult posterior midgut.

Fluorescent dyes

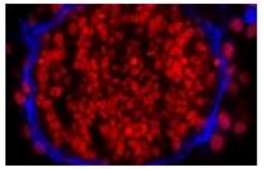
Combine fluorescent proteins with fluorescent dyes



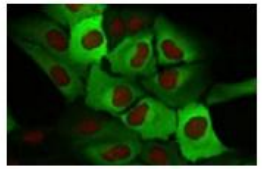
DRAQ5™ (ab108410)
Nuclear stain for live and fixed cells. No photobleaching effect.



Nuclear Green DCS1 (ab138905)
DNA selective dye for dead/fixed or apoptotic cells.



DRAQ7™ (ab109202)
Nuclear stain for **dead** and permeabilized cells.



CyTRAK Orange™ (ab109203)
Nuclear and cytoplasmic staining for live and fixed cells.

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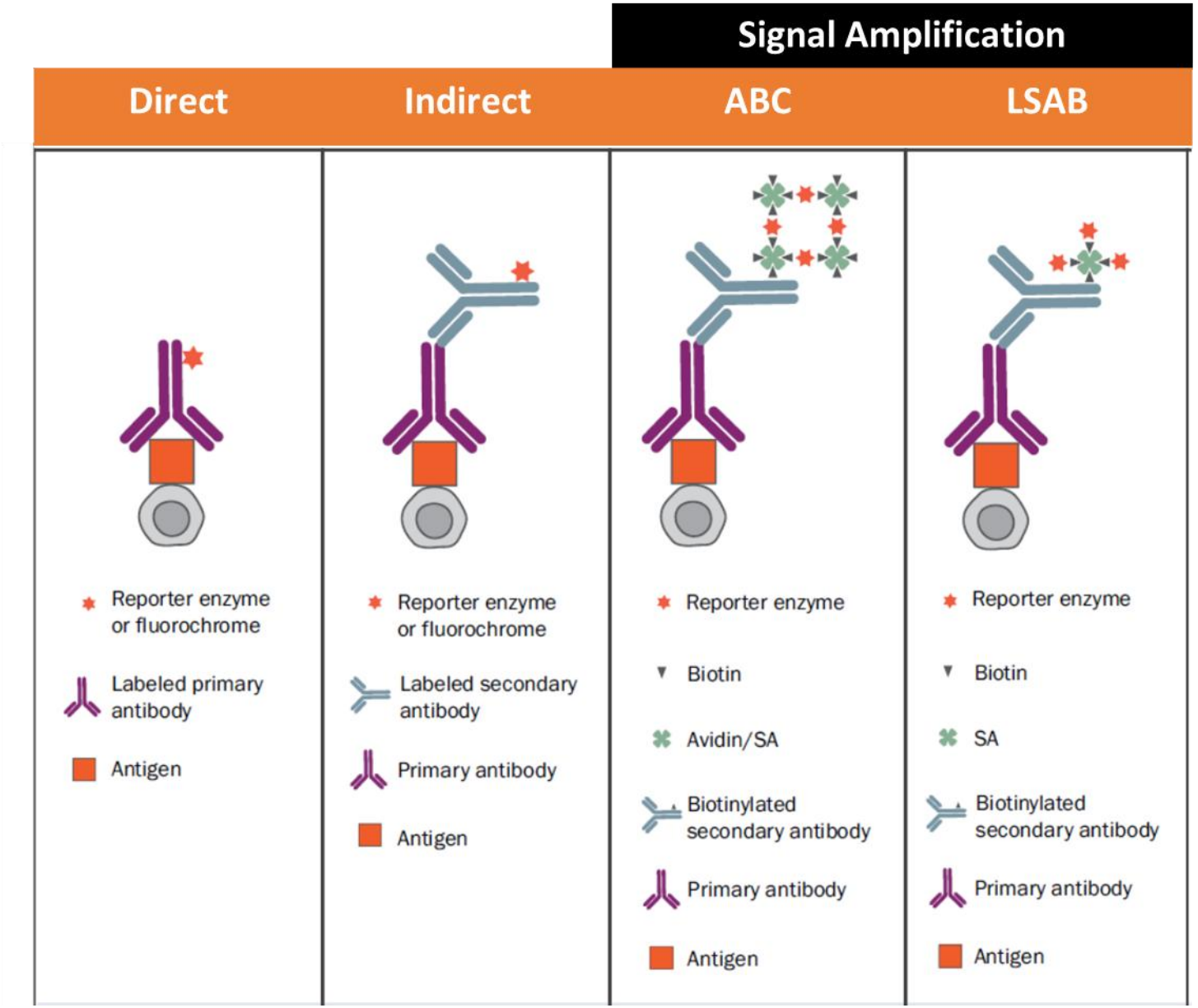
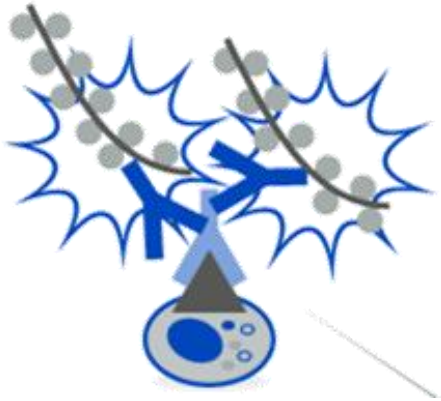
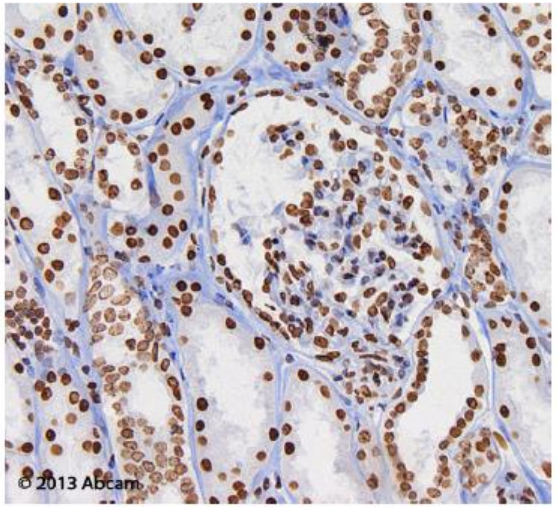

Signal Amplification
Direct
Indirect
ABC
LSAB
Reporter enzyme or fluorochrome
Labeled primary antibody
Antigen
Reporter enzyme or fluorochrome
Labeled secondary antibody
Primary antibody
Antigen
Reporter enzyme
Biotin
Avidin/SA
Biotinylated secondary antibody
Primary antibody
Antigen
Reporter enzyme
Biotin
SA
Biotinylated secondary antibody
Primary antibody
Antigen

Micro-polymer IHC Detection Kit

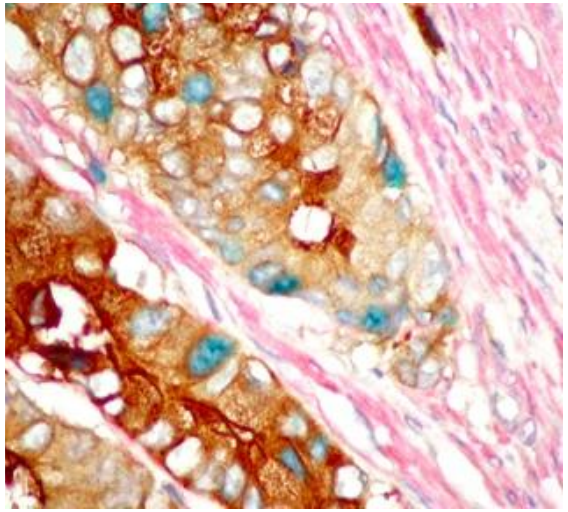


- **Increased sensitivity (penetrate tissues better)**
- **No background from endogenous biotin**
- **Time saving: only 2 steps**

Cat.	Product name
ab236466	Mouse and Rabbit Specific HRP/DAB IHC Detection Kit - Micro-polymer
ab236467	Mouse and Rabbit Specific HRP/AEC IHC Detection Kit - Micro-polymer
ab183286	TripleStain IHC Kit: M&M&R on Human tissue (DAB, AP/Red & HRP/Green)



IHC-P image generated with Micro-polymer IHC kit (ab236466). Human kidney sections stained by Anti-Histone H3 (di methyl K9) antibody (ab1220).



IHC-P with TripleStain Kit. Human colon cancer tissue. Mouse anti-smooth muscle actin (red), rabbit anti-CEA (brown), mouse anti-PCNA (emerald).

伯森生技

Counterstains

→ To aid localization of your primary antibody

Type	Dye	Target	Color
Chromogenic	Hematoxylin	Nuclei	Blue to violet
Chromogenic	Nuclear fast red (Kernechtrot)	Nucleic acids	Red
Chromogenic	Methyl green	Nucleic acids	Green
Fluorescent	Nuclear Green DCS1	Nucleic acids	Green
Fluorescent	Hoechst stain	Nucleic acids	Blue
Fluorescent	DAPI	Nucleic acids	Blue
Fluorescent	Propidium iodide	Nucleic acids	Red

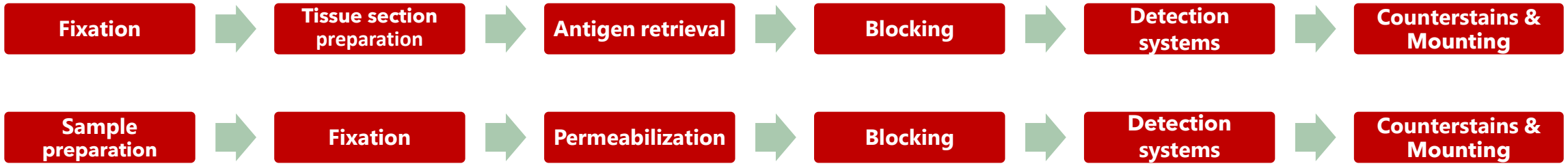
Mounting

→ preserving the specimen during storage & enhancing imaging quality during microscopy

— **Aqueous:**
Fluorescent labels
(Anti-Fade Fluorescence Mounting Medium is recommended)
Some Enzymatic labels (fast red, AEC)

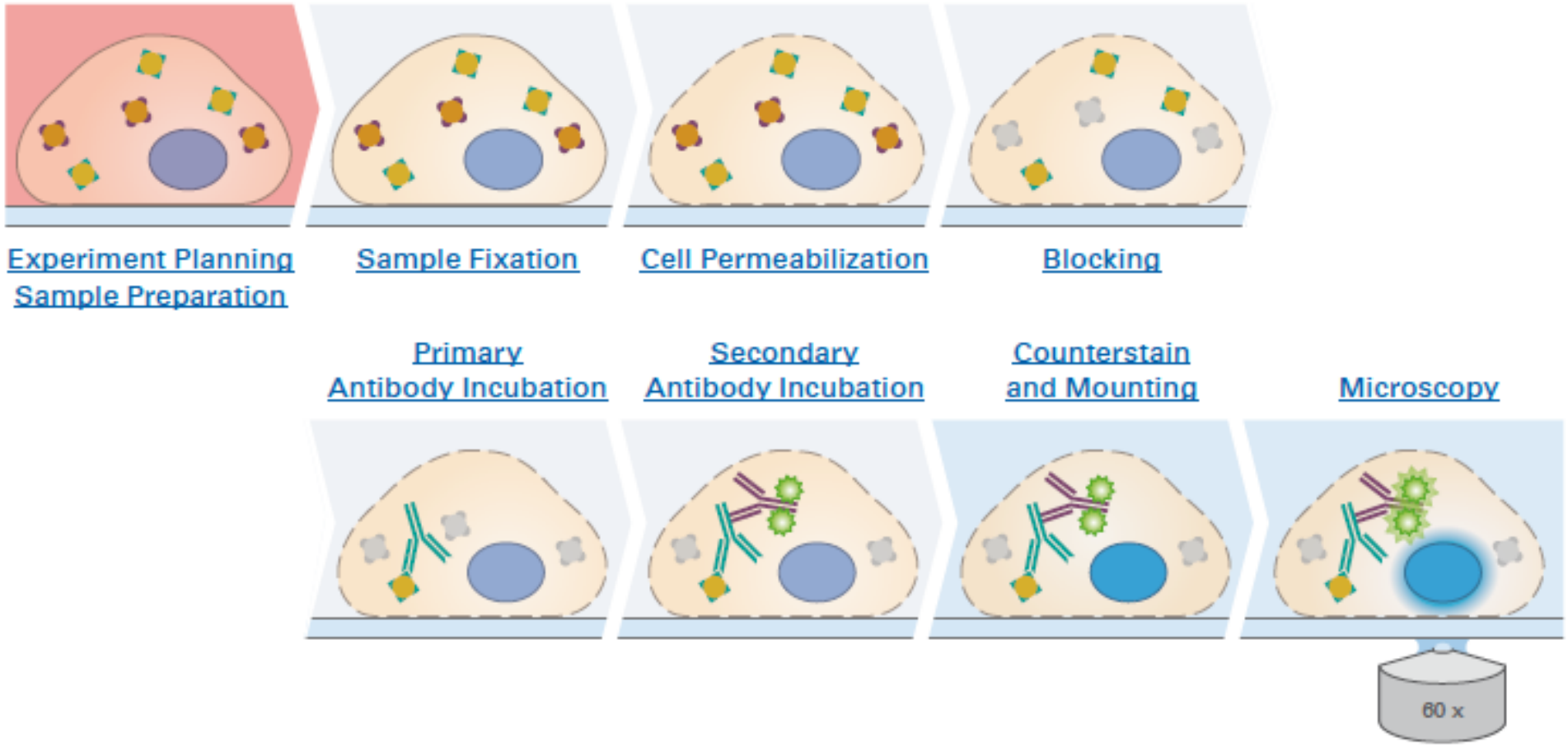
— **Organic:**
Only for enzymatic
(Refractive indexes are better with organic mounting media, which give sharper images.)

Tissue sample staining



ICC/IF staining

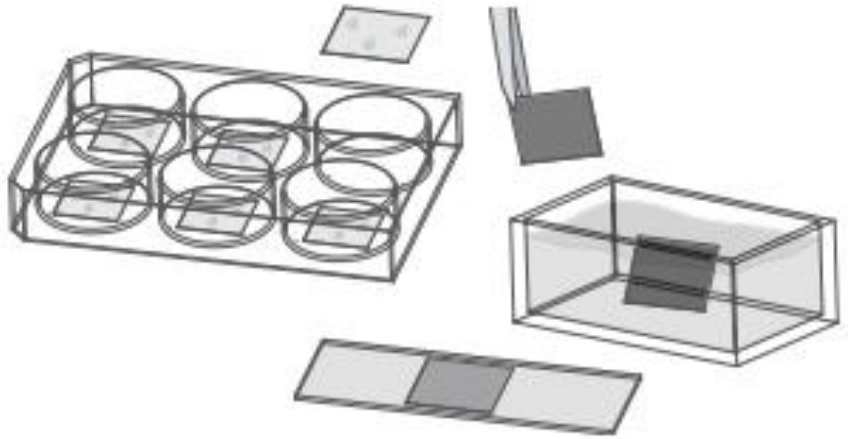
Immunocytochemistry-Immunofluorescence (ICC/IF) Workflow

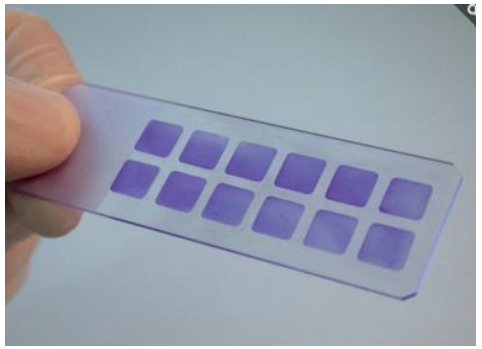


伯森生技

Cell preparation

- Adhesion cells
  - Poly-L-lysine / Poly-D-lysine / gelatin coated coverslips
  - Chamber slides

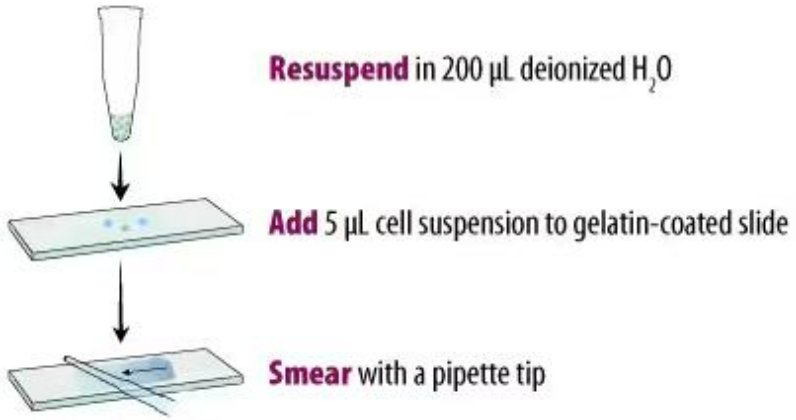




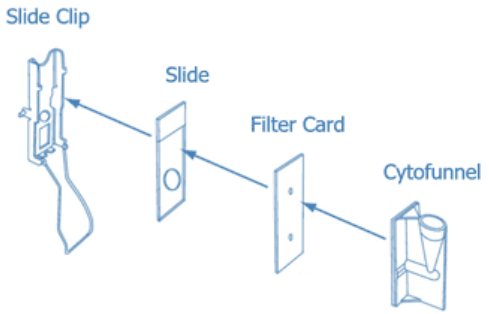
Cell preparation

- Non-adhesion cells
  - Cell Smear
  - Cytospin

Cell Smear



Cytospin



伯森生技

Fixative

Fixative	Conditions
4% paraformaldehyde (PFA) in PBS	Incubate for 10 – 30 min at RT
Methanol (95-100%)	Incubate for 5 – 10 min at -20°C
Ethanol (95-100%)	Incubate for 5 – 10 min at -20°C
Acetone	Incubate for 5 – 10 min at -20°C

Fixative	Mechanism	Advantages	Disadvantages
Aldehyde Fixatives Formaldehyde, Glutaraldehyde	Protein cross-linking	Excellent preservation of morphology Recommended standard fixative	**Some antibody epitopes may be modified by cross-linking** Over-fixation lead epitopes masked by cross-linking and reducing antibody binding.
Alcoholic Fixatives Acetone, Ethanol, Methanol	Precipitants, coagulants	Good when epitope is masked by PFA fixation Preservation of cellular architecture (cytoskeletal markers) Do not need permeabilization	Some antibody epitopes may be modified by precipitation **May change membrane protein structure as dissolves lipids** Causes cell shrinkage and structural changes **Not recommended for overexpressed fluorescent proteins**

Detergents	Suggested concentration	Directions
Harsh detergents: Triton X-100, NP-40	0.1 – 0.5% in PBS	Incubate for 2 – 5 min
Mild detergents: Tween 20, saponin, digitonin	0.2 – 0.5% in PBS	Incubate for 2 – 5 min

Blocking

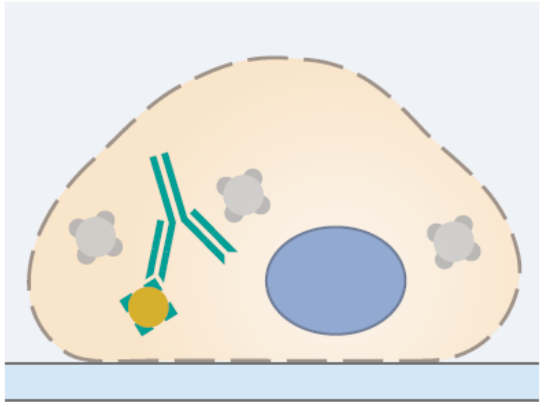
→ Preventing Non-Specific Signals

1. **Protein blocking with BSA / Normal serum**
2. ~~**Endogenous biotin**~~
3. ~~**Endogenous enzymes**~~
4. **Autofluorescence from PSA fixation: Glycine**

Antibody selection for double staining

Direct staining (primary antibodies)

- Antibody specificity
- Fluorescence dye selection

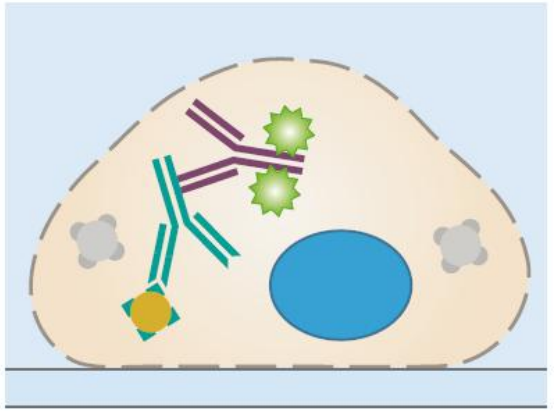


Indirect staining (primary antibodies)

- Antibody specificity
- Different host (mouse, rabbit…)
- Different isotype (IgG1, IgG2, IgM…)

Indirect staining (secondary antibodies)

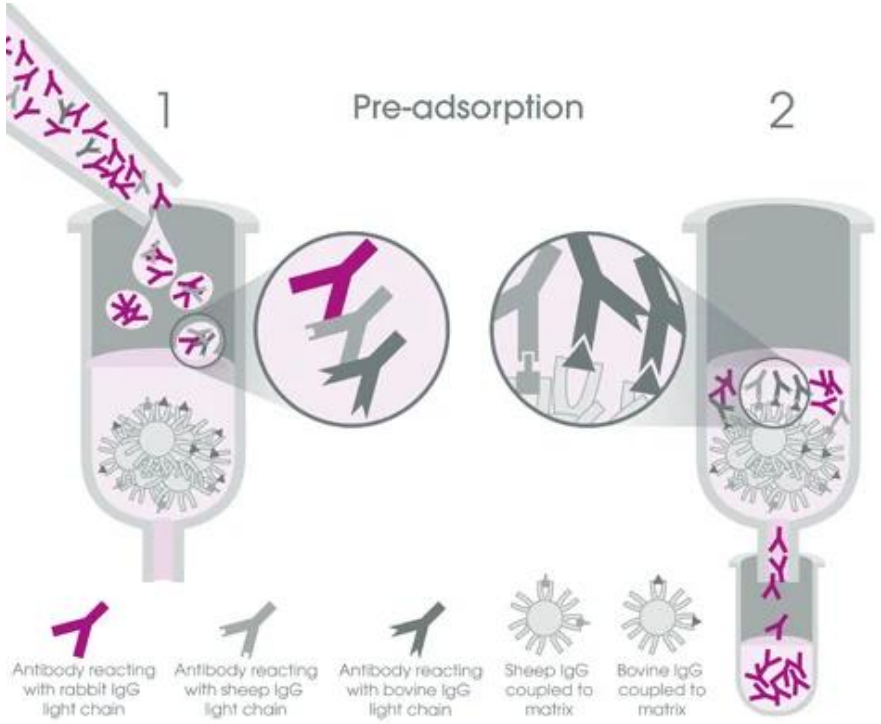
- Fluorescence dye selection
- Pre-adsorbed secondary antibody

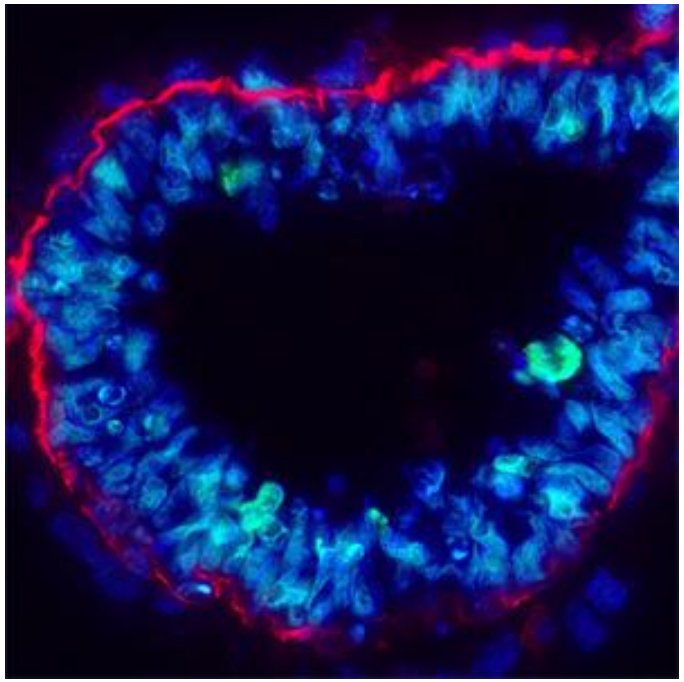


Pre-adsorbed secondary antibody

→Minimize non-specific binding and high background staining

- **Multicolor experiments**
- **Samples with abundant amounts of endogenous immunoglobulins (Igs)**





Sample: mouse embryonic stem cell-differentiated embryoid bodies (EBs)

Goat anti-Mouse – (Alexa Fluor® 488) pre-adsorbed (ab150117)

+

Goat anti- Rabbit – (Alexa Fluor® 594) pre-adsorbed (ab150084)

Counterstains

→ To aid localization of your primary antibody

Fluorescent	Nuclear Green DCS1	Nucleic acids	Green
Fluorescent	Hoechst stain	Nucleic acids	Blue
Fluorescent	DAPI	Nucleic acids	Blue
Fluorescent	Propidium iodide	Nucleic acids	Red

Mounting

— **Aqueous:**
Fluorescent labels
(Anti-Fade Fluorescence Mounting Medium is recommended)

TROUBLESHOOTING

- **Q1. No signals or low signals.**
- **Q2. High Background or Non-specific signals.**
- **Q3. Morphology Destroyed.**

伯森生技

TROUBLESHOOTING

- **Q1. No signals or low signals.**

1. Not enough primary antibody is bound to the protein of interest.
-- higher concentration of antibody.
-- Incubate longer (e.g., overnight) at 4°C.

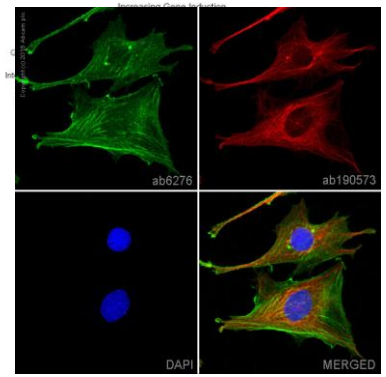
2. The antibody may not be suitable for IHC / IF procedures as it may not recognize the native (3D) form of the protein.
-- **Confirm the QC data to confirm the application of the antibody (Cell or Tissue?)**

3. The protein of is not present or not abundant in the tissue.
-- Run a positive control
-- Use an amplification step to maximize the signal.

4. Fixation procedures may be modifying the epitope that the antibody recognizes.
-- Use different antigen retrieval methods
-- Fix the sections for a shorter time.

伯森生技

Select correct antibody for the applications



ab6276

Anti-beta Actin antibody [AC-15] - Loading Control

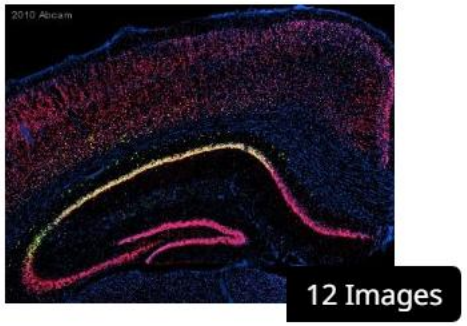
Lab Essentials | KO Validated | Recombinant

What is this?

5 ★★★★★ (94 Reviews)

2378 Publications

Target	ACTB
Applications	WB, ICC/IF
Reactivity	Mouse, Rat, Cow, Dog, Human, African green monkey, Chinese hamster
Host species	Mouse



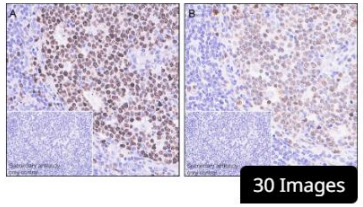
ab290

Anti-GFP antibody

5 ★★★★★ (179 Reviews)

3369 Publications

Target	GFP
Applications	IP, IHC-Fr, IHC-FrFl, IHC-P, EM, WB, ELISA, ICC/IF, IHC-FoFr
Host species	Rabbit



ab16667

Anti-Ki67 antibody [SP6]

KO Validated | RabMAb | Advanced Validation | Recombinant

What is this?

5 ★★★★★ (130 Reviews)

2747 Publications

Target	MKI67
Applications	mIHC, ICC/IF, IHC-P, Flow Cyt (Intra), WB
Reactivity	Mouse, Rat, Human
Host species	Rabbit

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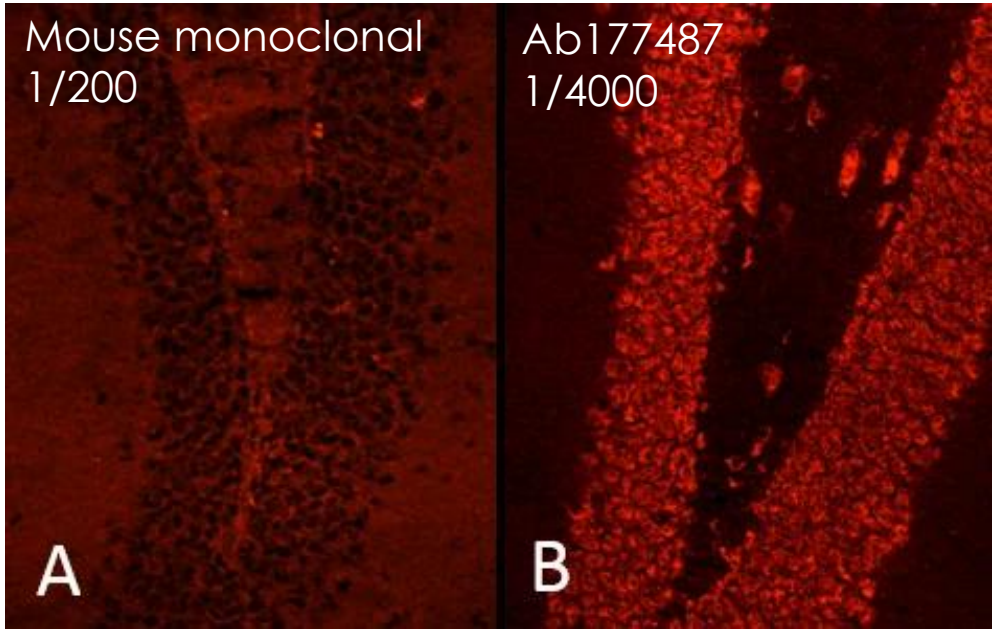
Recombinant RabMAb antibodies

-Best-In-Class IHC/ICC markers

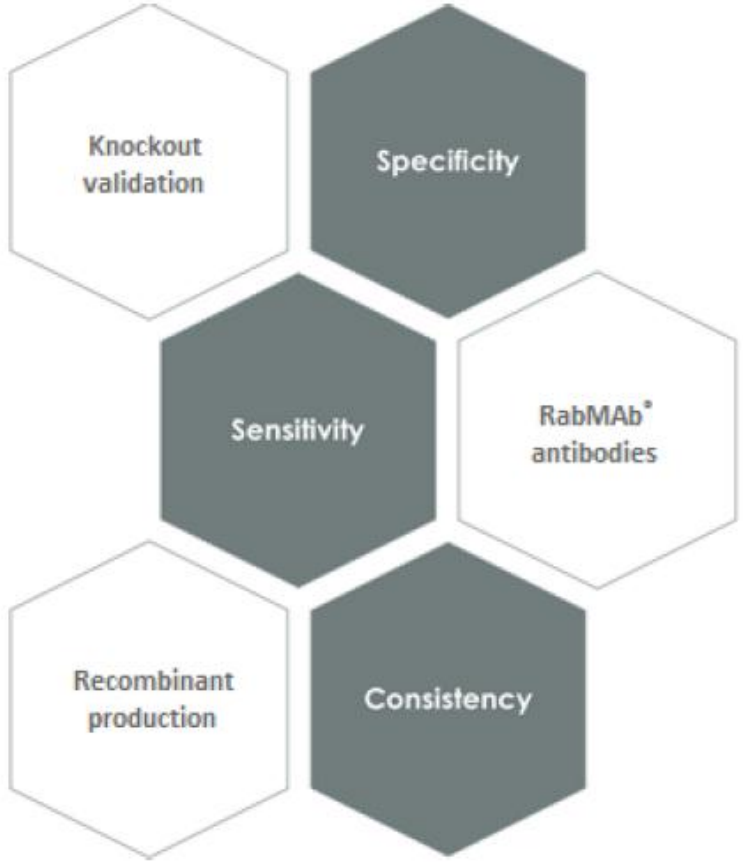
abcam

KO VALIDATED Recombinant RabMAb

NeuN antibodies



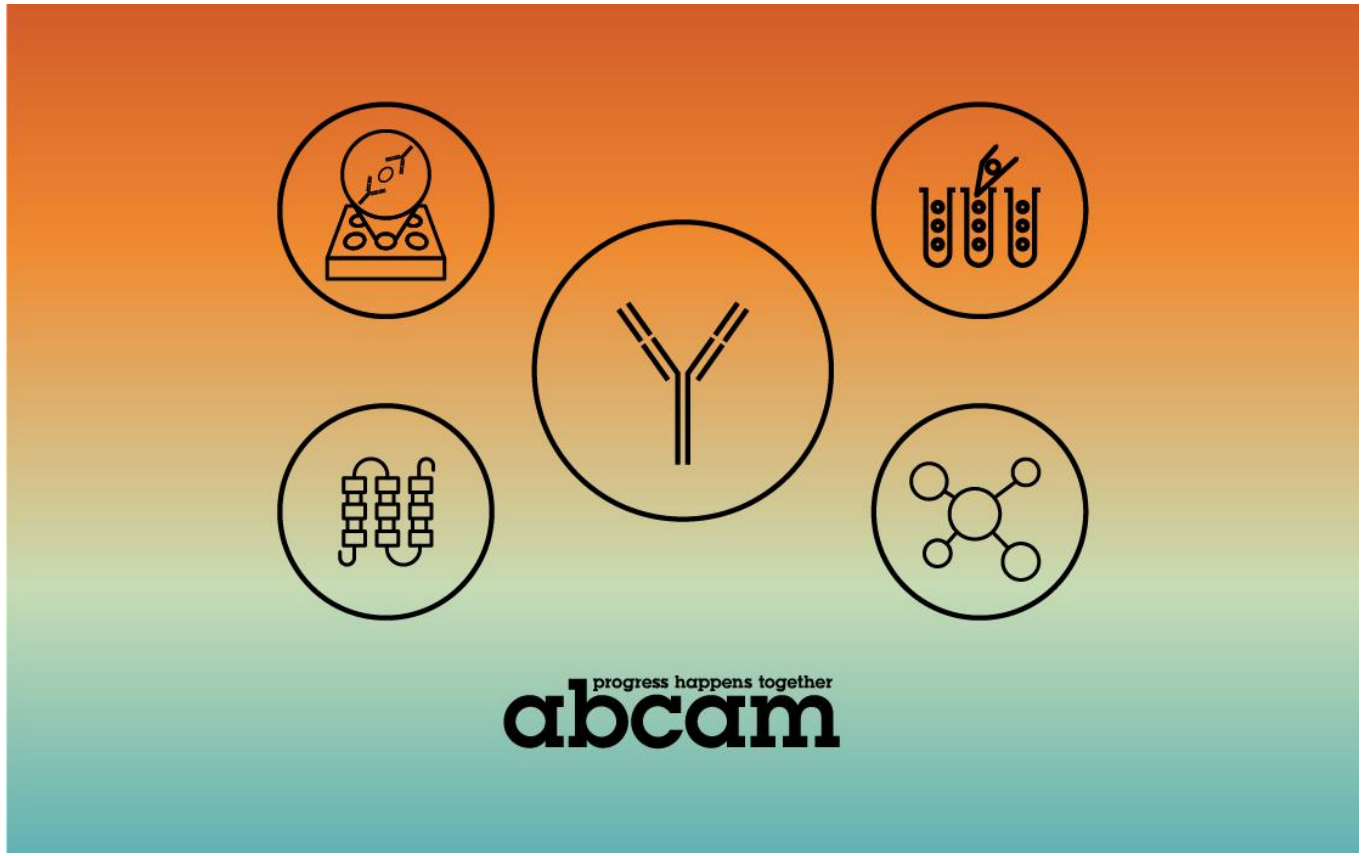
A comparison of leading NeuN antibodies on acetone-fixed adult mouse dentate gyrus frozen sections. A) Leading mouse monoclonal, 1/200 dilution, shows background that cannot be reduced by antibody dilution. B) Anti-Neun-antibody [ERP12763] (ab177487), 1/4000 dilution, shows specific staining with minimal background.



伯森生技

Abcam 產品無憂測試專案
— Abcam Trial Program —

- 想測試的樣本物種或實驗應用未列於該產品官網說明頁面，即可申請測試。
- 測試完成後，無論結果好壞，只要填寫產品測試結果，即可獲得產品等值的回饋折扣碼，在下筆 Abcam 訂單中折抵使用



伯森生技

TROUBLESHOOTING

- **Q1. No signals or low signals.**

1. Not enough primary antibody is bound to the protein of interest.
-- higher concentration of antibody.
-- Incubate longer (e.g., overnight) at 4°C.

2. The antibody may not be suitable for IHC procedures as it may not recognize the native (3D) form of the protein.
-- Confirm the QC data to confirm the application of the antibody

3. The protein of is not present or not abundant in the tissue/cell.
-- Run a positive control, check reference or database
-- Use an amplification step to maximize the signal. (PSA)

4. Fixation procedures may be modifying the epitope that the antibody recognizes.
-- Use different antigen retrieval methods
-- Fix the sections for a shorter time.

伯森生技

TROUBLESHOOTING

- **Q1. No signals or low signals.**

1. Not enough primary antibody is bound to the protein of interest.
-- higher concentration of antibody.
-- Incubate longer (e.g., overnight) at 4°C.

2. The antibody may not be suitable for IHC procedures as it may not recognize the native (3D) form of the protein.
-- Confirm the QC data to confirm the application of the antibody

3. The protein of is not present or not abundant in the tissue.
-- Run a positive control, check reference or database
-- Use an amplification step to maximize the signal.

4. Fixation procedures may modify the epitope that the antibody recognizes.
-- Use different antigen retrieval methods
-- Fix the sections for a shorter time.
-- Change fixative.

伯森生技

TROUBLESHOOTING

- **Q2: High Background or Non-specific signals.**

1. Blocking of non-specific binding might be absent or insufficient.

-- Increase blocking time

-- Tissue may have high autofluorescence, biotin, peroxidase activity, etc.

-- **Use the pre-adsorbed secondary antibody**

2. Permeabilization has damaged the membrane and removed the membrane protein.

-- Use a less stringent detergent (e.g., Tween 20 instead of Triton™ X-100).

-- Remove permeabilizing agent from your buffers.

3. The primary antibody is raised against the same species as the tissue stained

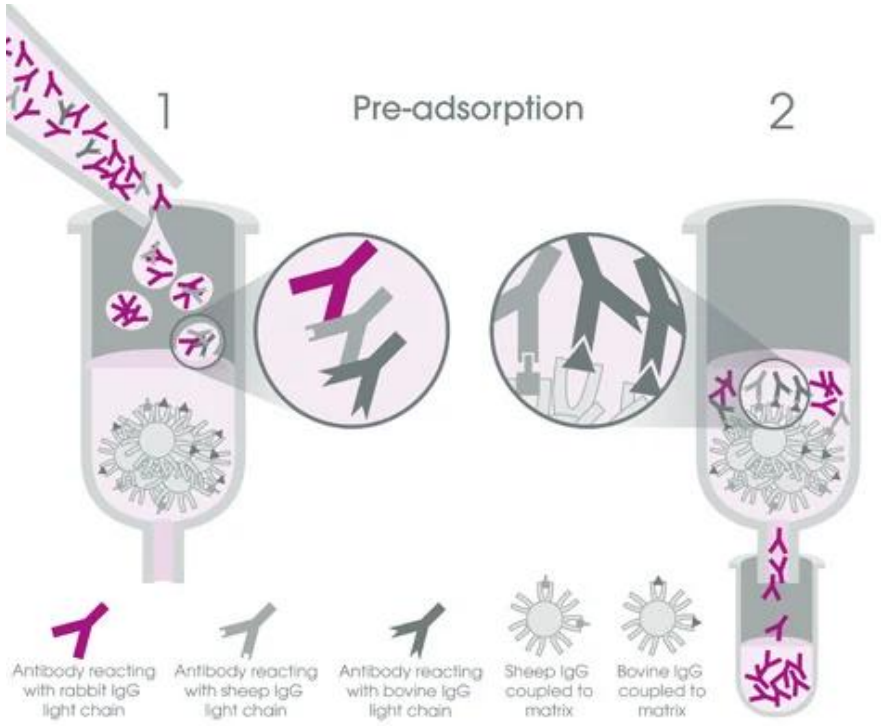
--Mouse on mouse kit

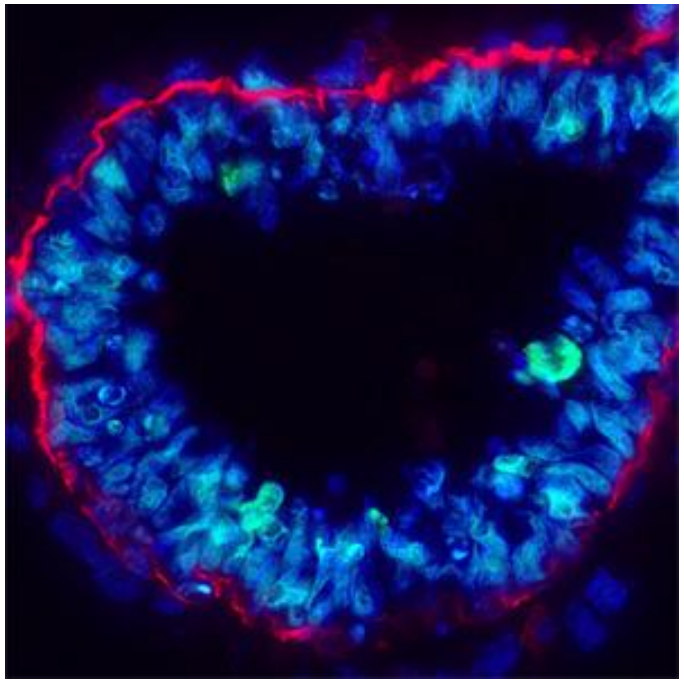
伯森生技

Pre-adsorbed secondary

→Minimize non-specific binding and high background staining

- **Multicolor experiments**
- **Samples with abundant amounts of endogenous immunoglobulins (Igs)**





Sample: mouse embryonic stem cell-differentiated embryoid bodies (EBs)

Goat anti-Mouse – (Alexa Fluor® 488) pre-adsorbed (ab150117)

+

Goat anti- Rabbit – (Alexa Fluor® 594) pre-adsorbed (ab150084)

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-- Remove permeabilizing agent from your buffers.

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-- Use a less stringent detergent (e.g., Tween 20 instead of Triton™ X-100).
-- Remove permeabilizing agent from your buffers.

3. The primary antibody is raised against the same species as the tissue stained
-- Mouse on mouse kit

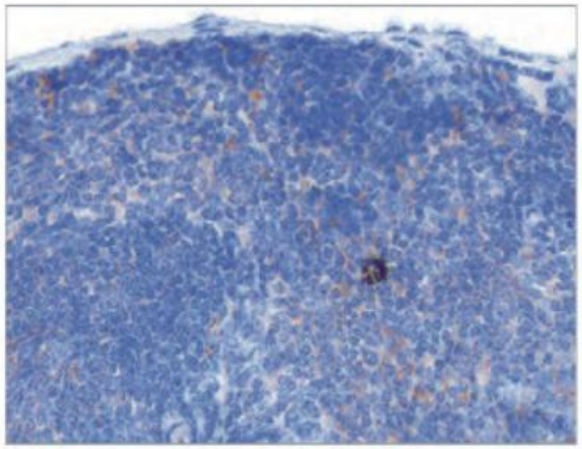
伯森生技

Mouse on Mouse Polymer IHC Kit (ab269452)

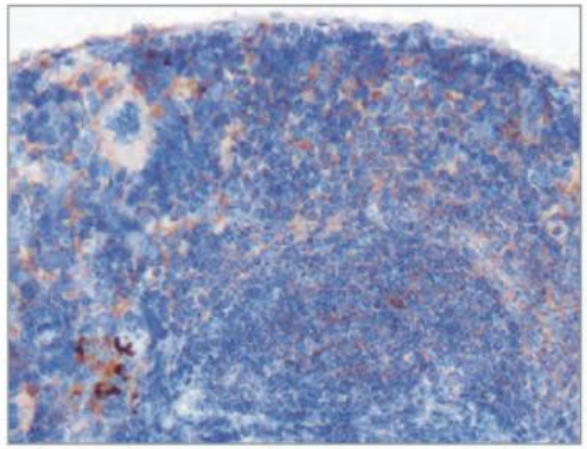
→Allows the use of mouse antibodies on mouse tissue

- **Clean background and clear signal**
- **Rodent blocking reagent：F(ab) fragment of an anti-mouse / anti-rat secondary antibody**.

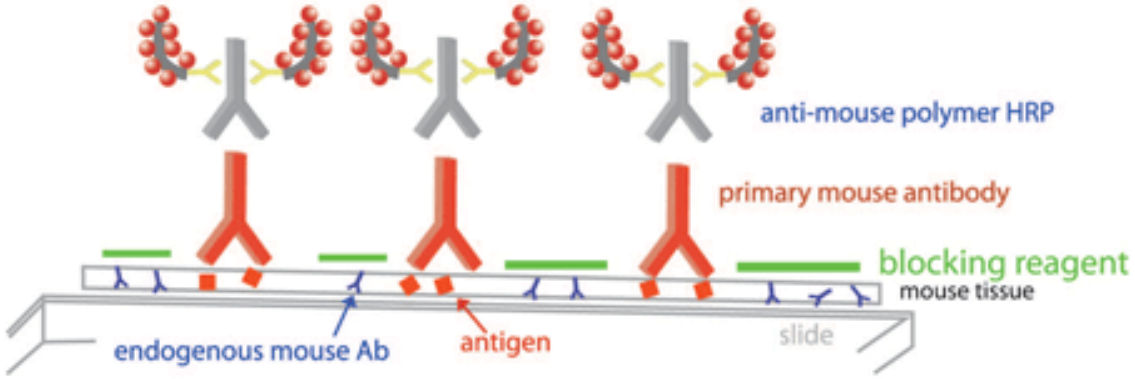
Reduced endogenous mouse IgG background using ab269452 - Both images show mouse spleen FFPE tissue.



Negative control images using Mouse on Mouse Polymer IHC kit (ab269452)



Negative control images using EXPOSE Mouse and Rabbit Specific HRP/DAB Detection IHC Kit(ab80436)



伯森生技

TROUBLESHOOTING

- **Q3: Morphology Destroyed.**

1. Antigen retrieval methods may be too harsh.

-- Vary antigen retrieval procedure or try different antigen retrieval methods.

2. The tissue may have been underfixed. (Autolysis or bacterial attack)

-- Increase fixation time.

-- Increase ratio of fixative to tissue.

-- Cut smaller pieces of tissue for more efficient fixation (fixation by immersion).

3. Tissue sections are falling off the slide (frozen sections).

-- Increase fixation time.

--Try alternative AR method.

-- Use freshly prepared slides.

4. Make sure cell not too dry, fixation time not too long

伯森生技

Antigen retrieval reagents

→To optimize your AR step and consistent buffer

Heat-induced (HIER)

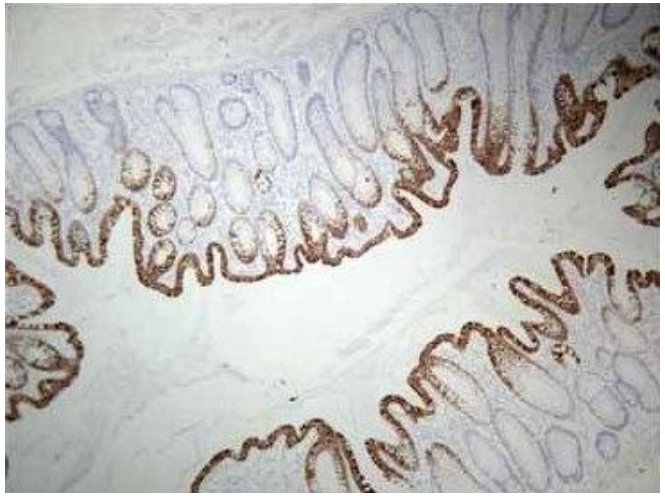
Cat.	Product name
ab93678	Antigen Retrieval Buffer (100X Citrate Buffer pH 6.0)
ab93684	Antigen Retrieval Buffer (100X Tris-EDTA Buffer, pH 9.0)
ab93682	Antigen Retrieval Buffer (100X Tris Buffer, pH 10.0)

Proteolytic-induced (PIER)

Cat.	Product name
ab64220	Proteinase K Antigen Retrieval Solution
ab970	Trypsin Antigen Retrieval Solution
ab64201	Pepsin Solution (Antigen Retrieval)
ab103720	**HistoReveal**

HistoReveal (ab103720)

- Gentle and does not destroy morphology
- 5 min
- Room temperature



Cytokeratin 20 being stained in colon tissue following antigen retrieval using HistoReveal.

伯森生技

TROUBLESHOOTING

- **Q3: Morphology Destroyed.**

1. Antigen retrieval methods may be too harsh.
-- Vary antigen retrieval procedure or try different antigen retrieval methods.

2. The tissue may have been underfixed. (Autolysis or bacterial attack)
-- Increase fixation time. (18-48 hr)
-- Increase the ratio of fixative to tissue. (1:10 – 1:50)
-- Cut smaller pieces of tissue for more efficient fixation (fixation by immersion).

3. Tissue sections are falling off the slide (frozen sections).
-- Increase fixation time.
--Try alternative AR method.
-- Use freshly prepared slides.

4. Make sure cell not too dry, fixation time not too long

伯森生技

TROUBLESHOOTING

- **Q3: Morphology Destroyed.**

1. Antigen retrieval methods may be too harsh.
-- Vary antigen retrieval procedure or try different antigen retrieval methods.

2. The tissue may have been underfixed. (Autolysis or bacterial attack)
-- Increase fixation time.
-- Increase ratio of fixative to tissue.
-- Cut smaller pieces of tissue for more efficient fixation (fixation by immersion).

3. Tissue sections are falling off the slide.
-- Increase fixation time.
-- Try alternative AR method and cool down slowly.
-- Use freshly prepared slides.
-- Make sure the slides are dry before storage, baking the slides before staining.

4. Make sure cell not too dry, fixation time not too long

伯森生技

TROUBLESHOOTING

- **Q3: Morphology Destroyed.**

1. Antigen retrieval methods may be too harsh.
-- Vary antigen retrieval procedure or try different antigen retrieval methods.

2. The tissue may have been underfixed. (Autolysis or bacterial attack)
-- Increase fixation time.
-- Increase ratio of fixative to tissue.
-- Cut smaller pieces of tissue for more efficient fixation (fixation by immersion).

3. Tissue sections are falling off the slide.
-- Increase fixation time.
-- Try alternative AR method and cool down slowly.
-- Use freshly prepared slides.
-- Make sure the slides are dry before storage, baking the slides before staining.

4. Make sure cell not too dry, fixation time not too long

伯森生技

SUMMARY

— The staining protocol needs to be **optimized** for each antigen and tissue type.

— Plan your experiment carefully:

1. Which **steps** do you want to test (antigen retrieval, dilution)?
2. What should the **ideal image** look like (location of protein)?
3. Identify the **controls** you need (isotype, no-primary, positive tissue)
4. Review the **references or protocol** before starting.

abcam product portfolio

A reagent portfolio that work the first time, every time

Recombinant Antibodies

Recombinantly manufactured antibodies for superior sensitivity, consistency between batches, and guaranteed long-term supply

ELISA and match antibody pairs

Extensive range enables flexible assay design and scalability

Conjugation ready antibody formats

Complete range of antibody conjugation solutions, including ready-to-use and bulk options, for flexibility without compr omising on experimental design

Biochemical and cell-based assays

Assays and kit components, available as ready-to-use and bulk options to adapt & scale

Conjugation kits and service

Quickly and easily achieve consistent conjugation at any scale

Cell engineering

Immediate access to >2.7k KO cell lysates and custom gene-edited cell lines

Bioactive Proteins

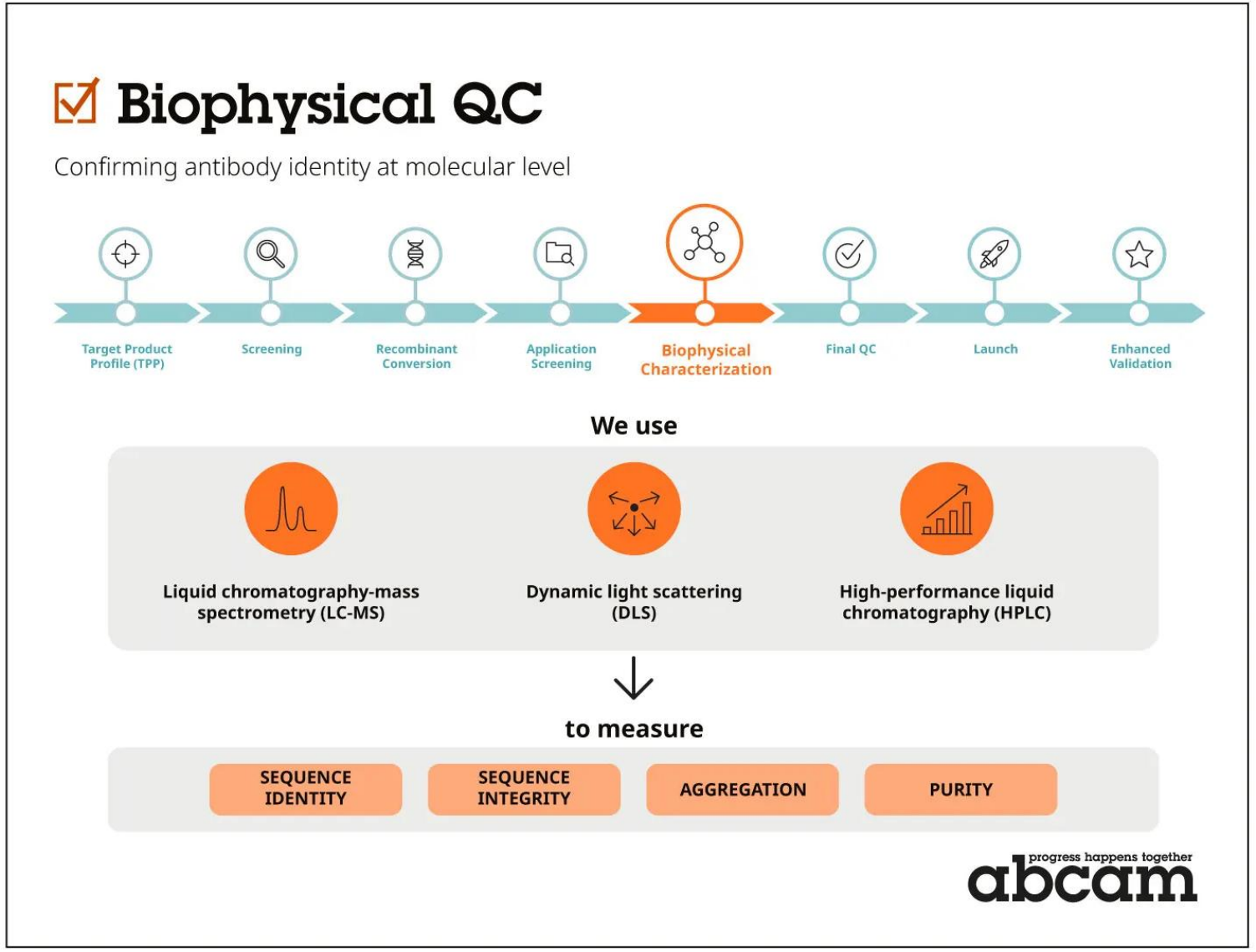
Extensive range of high-quality proteins with guaranteed batch-to-batch consistency to fit your needs and enable you to meet the challenges ahead with confidence

abcam

abcam antibodies for IHC & ICC/IF staining

- IHC validation on tissue microarrays (TMAs with 15 normal / 15 cancer types)
- Multiplex-ready antibodies (mIHC)
- Antibody optimization
- Recombinantly manufactured antibodies

Biophysical QC



Other validation techniques

Recombinant technology
Extensive application testing
(IHC included)
Advanced validation
Knock-out validation

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