Detection of proteins in polyacrylamide gel electrophoresis

- Electrophoresis is used to separate and analyze proteins based on their size and charge.

SDS PAGE	native PAGE
SDS; DTT or ß-ME	native protein

Polyacrylamide gel electrophoresis

Polyacrylamide gels are formed from the polymerization of two compounds, acrylamide and *N*,*N*'-methylenebisacrylamide (bis). The polymerization is initiated by the addition of ammonium persulfate (APS) & TEMED.

Protein size	Gel acrylamide percentage
4–40 kDa	20%
12–45 kDa	15%
10–70 kDa	12.5%
15–100 kDa	**10%**
25–200 kDa	8%

Sample preparation for electrophoresis

- Extraction & solubilization of a protein sample free of contaminants and has a total protein concentration suitable for electrophoresis.

Osmotic lysis

Freeze thaw lysis

Enzymatic lysis

Sonication

French press

Grinding

Mechanical homogenization

Glass bead homogenization

Gentler → Harsher

Detergents *& reducing agents*

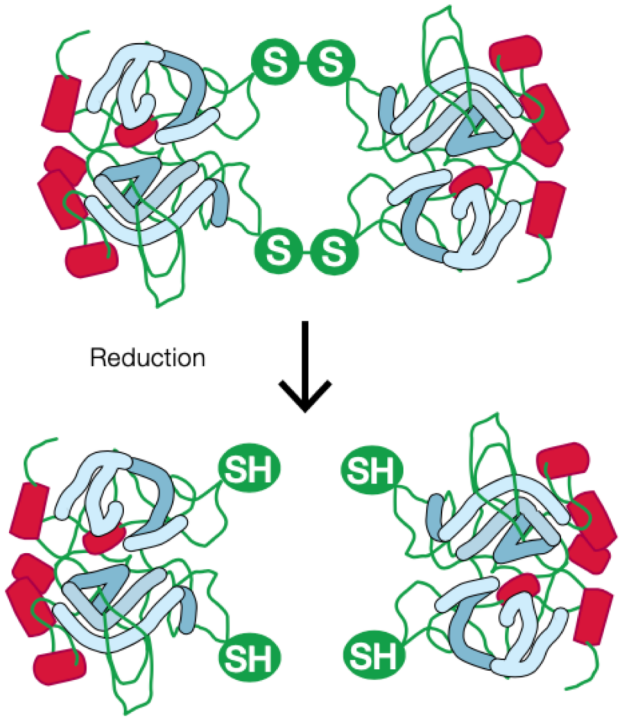
- Detergents disrupt hydrophobic interactions between & within proteins.
- SDS
  - NP-40
  - Triton X-100

  - CHAPS
  - Sulfobetaines (SB 3-10, ASB-14 etc.)

detergents & *Reducing agents*

- 2-Mercaptoethanol (ß-ME)
- Dithiotheritol (DTT)

- Tributylphosphine (TBP)
- Tris-carboxyethylphosphine (TCEP)



Protease inhibitors

Inhibitor

Sodium Fluoride
Sodium Orthovanadate
beta-Glycerophosphate (disodium salt)
Sodium Pyrophosphate
AEBSF•HCl
Aprotinin
Bestatin
E-64
EDTA
Leupeptin
Pepstatin A
PMSF

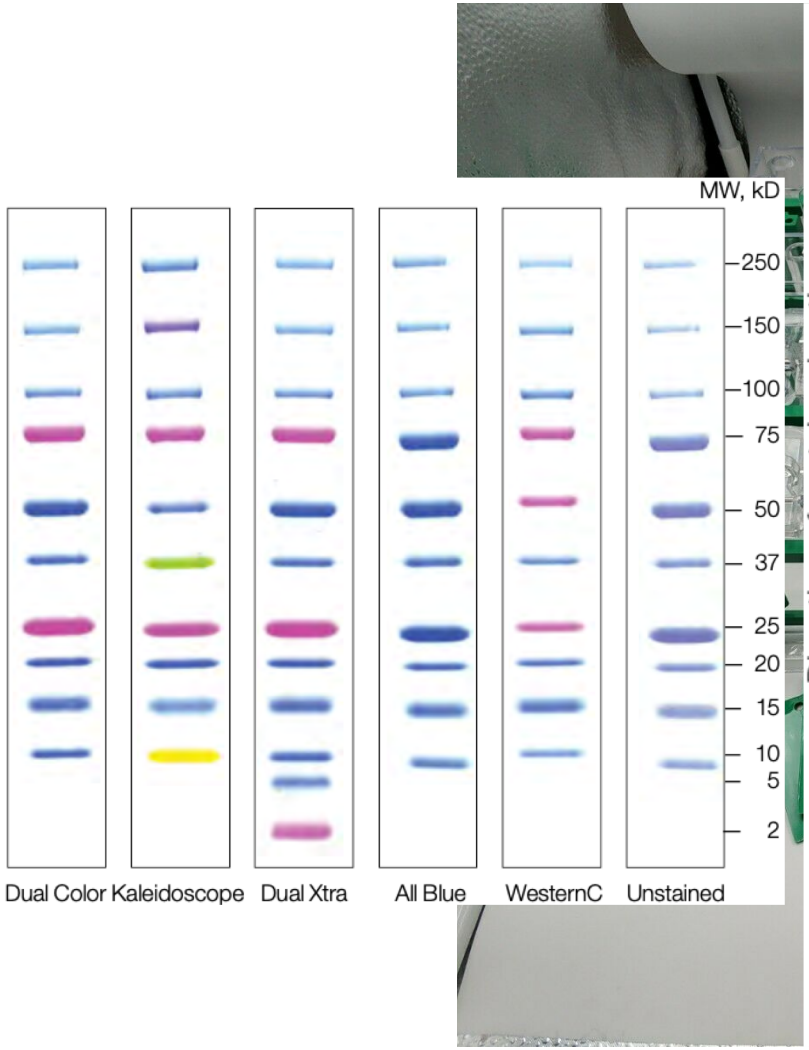
Sample quantification / Protein assays

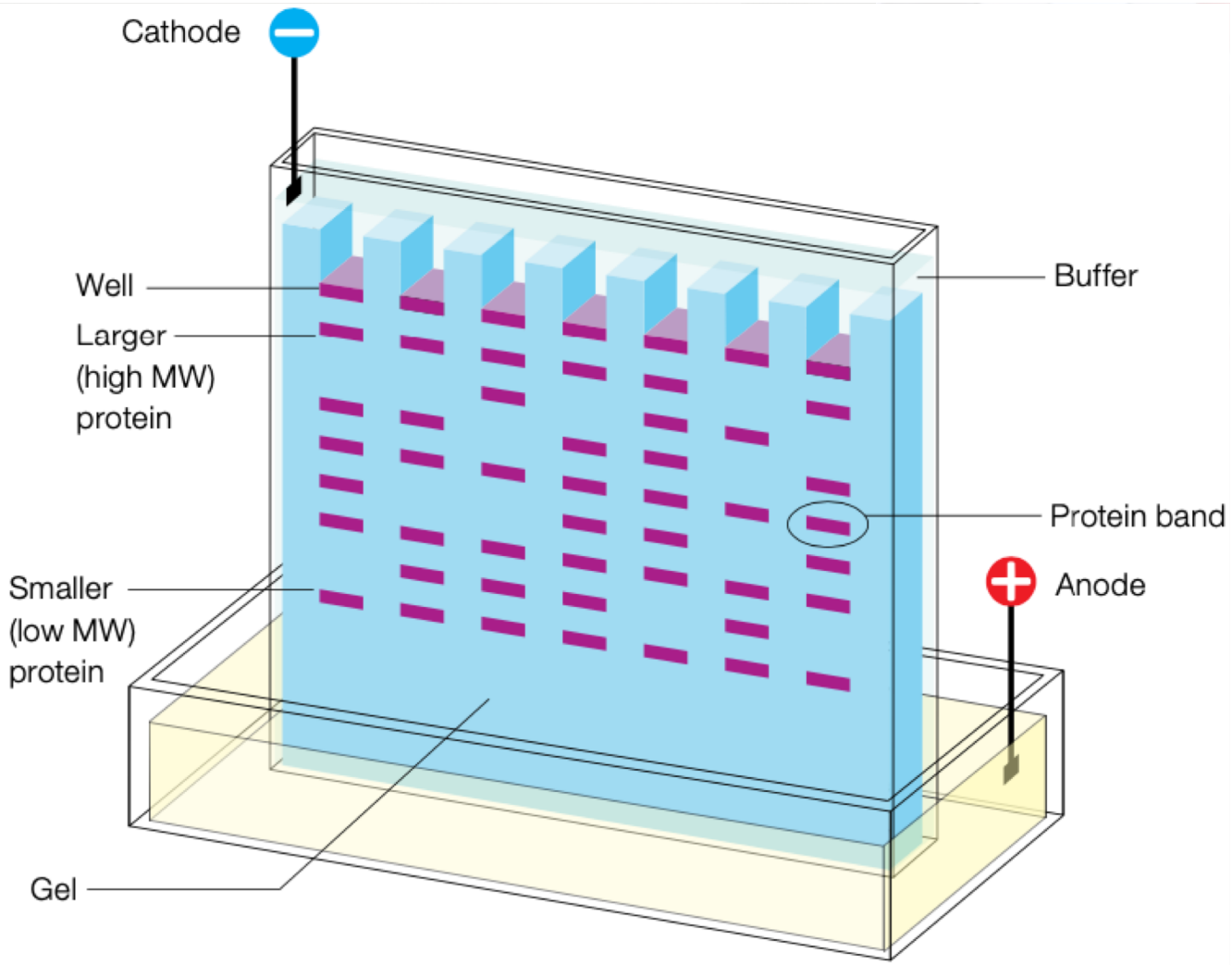
- To determine the concentration of protein in a sample.
- To ensure the amount of protein to be loaded for each lane.
- To compare among similar samples.

Colorimetric methods:

- Bradford assay
- Lowry assay
- BCA

Performing electrophoresis

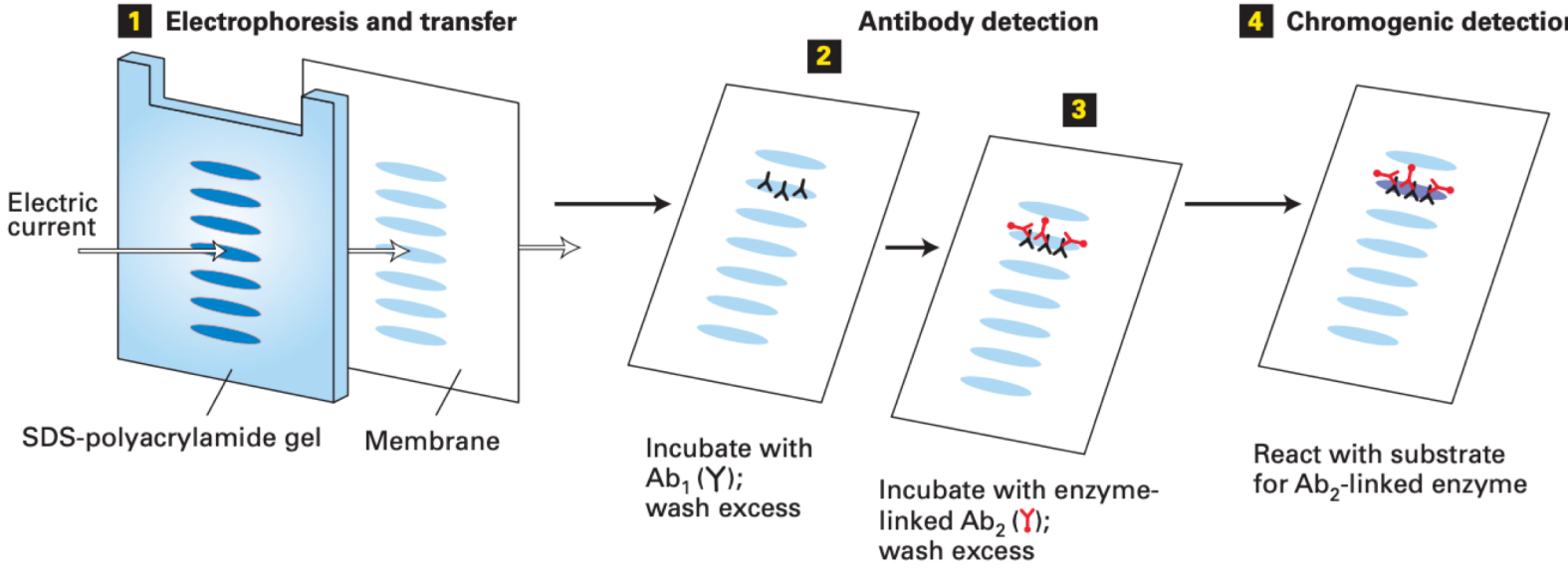


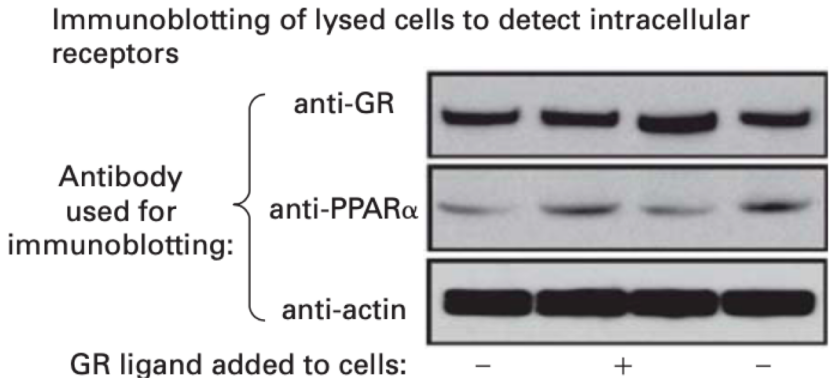


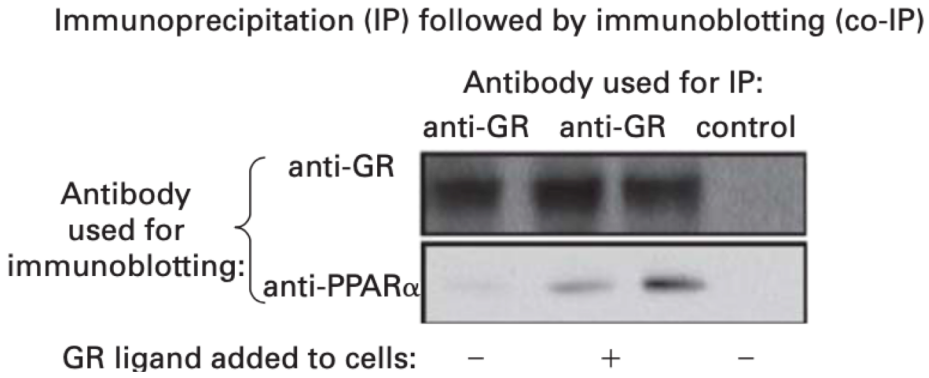
Staining Proteins in Gels

- Coomassie dye staining
- Silver staining
- Zinc staining
- Fluorescent dye staining

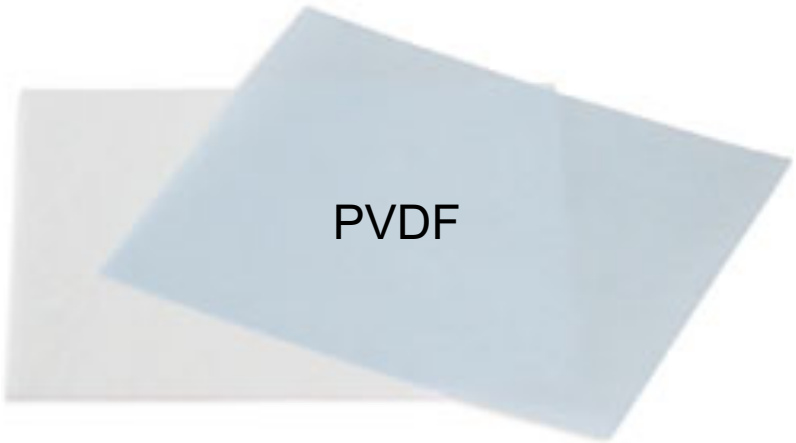
Western Blotting / Immunoblotting







Membranes in Western Blotting



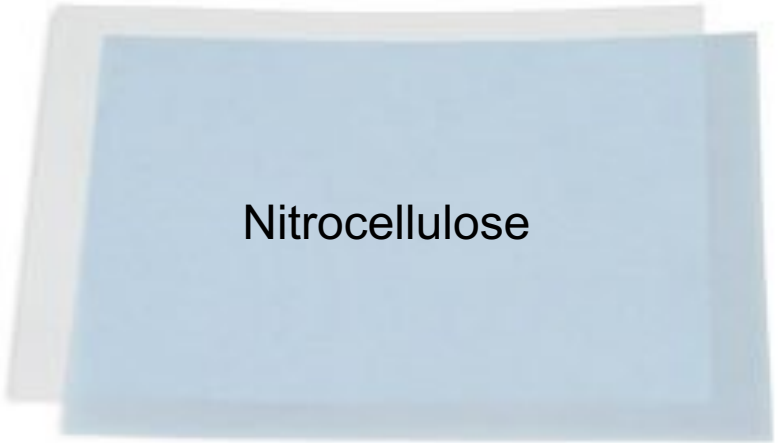
150-200 µg of protein/cm^2

0.2 µm and 0.45 µm pore size

less fragile

must be pre-wetted with methanol

chemiluminescence and fluorescence detection



80-100 µg of protein/cm^2

0.2 µm and 0.45 µm pore size

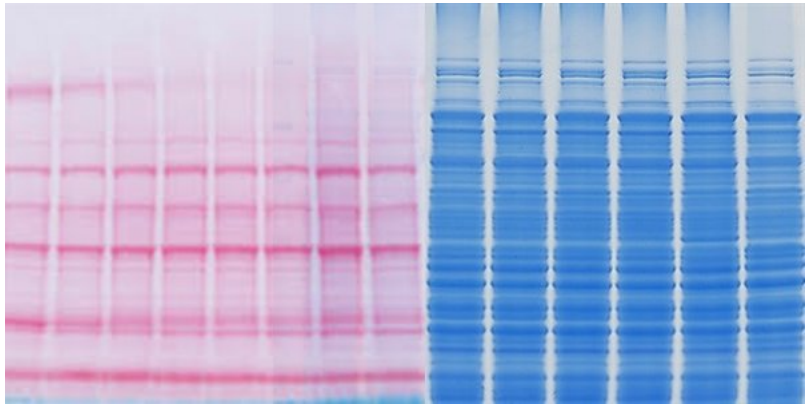
more fragile

transfer buffer must contain methanol

chemiluminescence and fluorescence detection

Staining Proteins in Membranes

- Ponceau S staining
- Coomassie blue R-250 staining
- Amido black staining
- Colloidal gold staining
- Colloidal silver staining
- India ink staining
- Memcode staining
- Fluorescamine staining

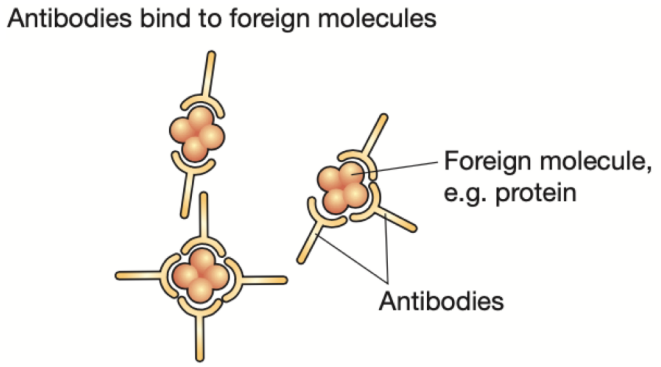


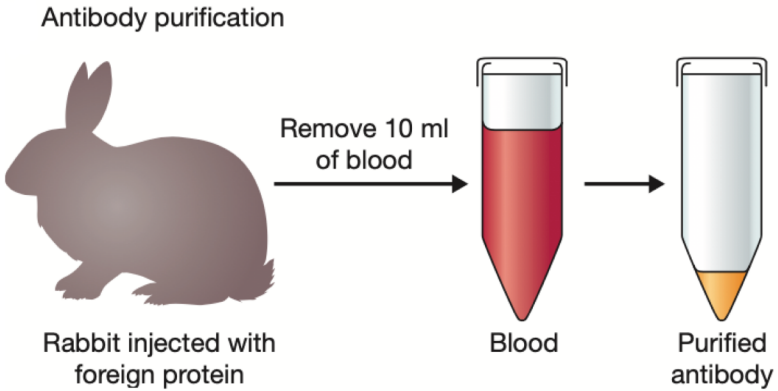
Ponceau Coomassie

Detecting proteins

- the bound antibody—the **primary antibody**—is detected by washing the membrane with a labeled **secondary antibody**, which binds specifically to the primary antibody.

Fluorescent
Chemiluminescent
Radioactive





Antibodies

- **Monoclonal antibodies**

  Monoclonal antibodies come from a single B-cell parent clone and therefore only recognize a single epitope per antigen.

- **Polyclonal antibodies**

  Polyclonal antibodies are a heterogeneous mix of antibodies, derived from the immune response of multiple B-cells, and each one recognizes a different epitope on the same antigen.

- **Recombinant antibodies**

  Monoclonal antibodies which are generated in vitro using synthetic genes using traditional hybridoma-based technologies.

Antibodies: real lab case

Product name	Catalog #	Supplier	Price
GPR56 Antibody (G-6) 200 µg/ml	sc-390192	Santa Cruz Biotechnology	$345.00
Gα q/11/14 Antibody (G-7) 200 µg/ml	sc-365906	Santa Cruz Biotechnology	$345.00
Gα 12 Antibody (B-5) 200 µg/ml	sc-515610	Santa Cruz Biotechnology	$345.00
Gα 13 Antibody (6F6-B5) 100 µg/ml	sc-293424	Santa Cruz Biotechnology	$345.00
β-Arrestin-1/2 Antibody (A-1) 200 µg/ml	sc-74591	Santa Cruz Biotechnology	$345.00
GFP Antibody (B-2) 200 µg/ml	sc-9996	Santa Cruz Biotechnology	$345.00
β-Actin Antibody (C4)	sc-47778	Santa Cruz Biotechnology	$325.00
m-IgGκ BP-HRP 200 µg/0.5 ml	sc-516102	Santa Cruz Biotechnology	$129.00

Anti-β-Arrestin-1/2 Antibody

- Anti-β-Arrestin-1/2 Antibody (A-1) is a mouse **monoclonal** IgG_1 κ β-Arrestin-1/2 antibody, cited in **16 publications**, provided at **200 µg/ml**
- raised against amino acids 7-290 mapping near the N-terminus of β-Arrestin-1 of human origin
- Anti-beta-Arrestin-1/2 Antibody (A-1) is recommended for detection of β-Arrestin-1 and β-Arrestin-2 of mouse, rat and human origin by WB, IP, IF, IHC(P) and ELISA; also reactive with additional species, including porcine
- Anti-beta-Arrestin-1/2 Antibody (A-1) is available conjugated to **agarose** for IP; **HRP** for WB, IHC(P) and ELISA; and to either **phycoerythrin** or **FITC** for IF, IHC(P) and FCM
- also available conjugated to **Alexa Fluor® 488**, **Alexa Fluor® 546**, **Alexa Fluor® 594** or **Alexa Fluor® 647** for WB (RGB), IF, IHC(P) and FCM, and for use with RGB fluorescent imaging systems, such as iBright™ FL1000, FluorChem™, Typhoon, Azure and other comparable systems
- also available conjugated to **Alexa Fluor® 680** or **Alexa Fluor® 790** for WB (NIR), IF and FCM; for use with Near-Infrared (NIR) detection systems, such as LI-COR®Odyssey®, iBright™ FL1000, FluorChem™, Typhoon, Azure and other comparable systems
- Contact our Technical Service Department (or your local Distributor) for more information on how to receive a **FREE 10 µg sample** of **β-Arrestin-1/2 (A-1): sc-74591**.
- **m-IgGκ BP-HRP (mouse IgGκ binding protein-HRP)** is the preferred secondary detection reagent for β-Arrestin-1/2 Antibody (A-1) for WB and IHC(P) applications. This reagent is now offered in a bundle with β-Arrestin-1/2 Antibody (A-1) (**see ordering information below**). For additional m-IgGκ BP conjugates see our complete list of **Mouse IgG Binding Proteins**.

Antibodies: Healthcare

Anti-IgG BP-HRP 200 µg/0.5 ml

- mouse IgG kappa binding protein (m-IgGκ BP) conjugated to Horseradish Peroxidase (HRP)
- supplied at 200 µg in 0.5 ml volume
- Highly recommended alternative to conventional anti-mouse IgG secondary antibodies for chemiluminescence Western blotting (WB [ECL]) and Immunohistochemistry (IHC)
- Suitable for binding to **mouse IgGκ light chain** immunoglobulins, comprising approximately **98%** of mouse monoclonal antibodies; not suitable for use with mouse monoclonal IgGλ light chain antibodies
- Highly specific reagent that provides strong signal with minimal background and virtually complete elimination of lot to lot variation associated with conventionally generated secondary antibodies
- For a Cruz Marker™ compatible mouse IgG kappa binding protein, use m-IgGκ BP-HRP (Cruz Marker) (sc-516102-CM) (Click here for datasheet)
- For mouse IgGλ immunoglobulins, comprising approximately **2%** of mouse monoclonal antibodies; we recommend m-IgGλ BP-HRP (sc-516132)
- Mouse IgG binding proteins are recommended for some, but not all of our monoclonal antibodies. **Product descriptions on our monoclonal antibody product pages will state if the Mouse IgG binding protein is the preferred detection reagent for that product.**
- Also see our new **m-IgGκ BP-FITC, PE, CFL 488, CFL 555, CFL 594, CFL 647, CFL 680 and CFL 790** fluorescent dye conjugates. These represent substantial improvements compared to conventional polyclonal anti-mouse secondary antibody fluorescent dye conjugates.

Detecting Proteins in Gels

kDa	Whole cell	Mitochondrial	Nuclear	Membrane	Cytoskeleton	Serum
125	Vinculin					
110				NaK ATPase		
75						Transferrin
66			Lamin B1			
60		HSP60				
55			HDAC1			
	Alpha tubulin				Alpha tubulin	
50	Beta tubulin				Beta tubulin	
			YY1			
	Actin				Actin	
40	Beta actin				Beta actin	
35	GAPDH		TBP			
30		VDAC1/Porin	PCNA			
	Cyclophilin B					
20	Cofilin	COX IV			Cofilin	
15			Histone H3			

Developing the antibodies