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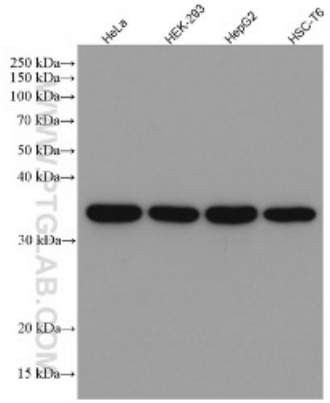
Perfect Your Western Blots with Proteintech Reagents

While western blotting appears technically simple, it can be daunting when it comes to generating perfect, crisp western blot images for publications. Fortunately, there is a lot of room to optimize your western blots which starts with the use of high-quality reagents for performing your experiments.

Proteintech reagents that can help you achieve publication worthy western blot results:

- ✓ Primary Antibodies
- ✓ Secondary Antibodies
- ✓ Secondary Nanobodies
- ✓ Tag/Control Antibodies
- ✓ Chemiluminescent Substrates
- ✓ Protein Ladders



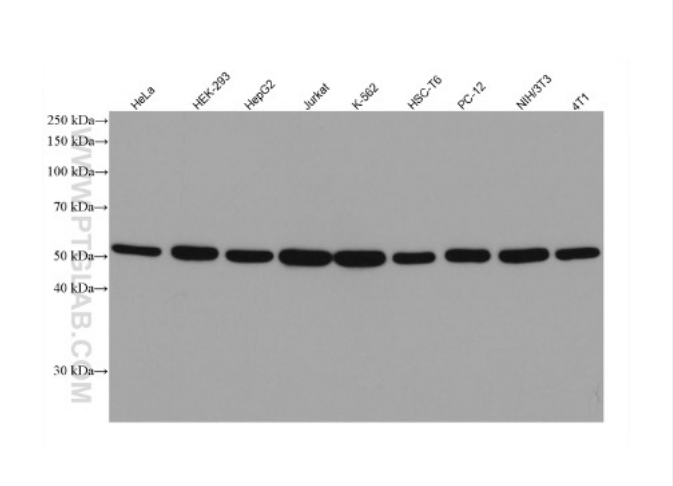
Primary Antibodies

- Rabbit polyclonal or mouse monoclonal antibodies
- Recombinant rabbit monoclonal antibodies
- Unconjugated antibodies
- CoraLite® dye or HRP-conjugated antibodies

Figure 1. Various lysates were subjected to SDS PAGE followed by western blot with 60004-1-Ig (GAPDH antibody) at dilution of 1:50,000 incubated at room temperature for 1.5 hours.

60004-1-Ig was cited in >5500 publications

Scan here to view our full selection of primary antibodies



Secondary Antibodies

- Polyclonal secondary antibodies conjugated to HRP or CoraLite® dyes
- Recombinant secondary antibodies - Coming soon!

Figure 2. Various lysates were subjected to SDS PAGE followed by western blot with 66031-1-Ig (alpha Tubulin antibody) at dilution of 1:100,000 incubated at room temperature for 1.5 hours. SA00001-1 (HRP-conjugated Affinipure Goat Anti-Mouse IgG(H+L)) was used as secondary antibody.

SA00001-1 was cited in >3300 publications

Scan here to view our full selection of secondary antibodies

Secondary Nanobodies

Nano-Secondaries® or Secondary Nanobodies are a novel class of secondary antibodies consisting of Nanobodies/ VHHs conjugated to fluorescent dyes. Due to their high isotype specificity and the absence of cross-reactivity, Secondary Nanobodies bind only to primary antibodies of a specific isotype. Even co-incubation of multiple primaries along with multiple Nano-Secondaries is possible making them highly suitable for multiplex western blot experiments.

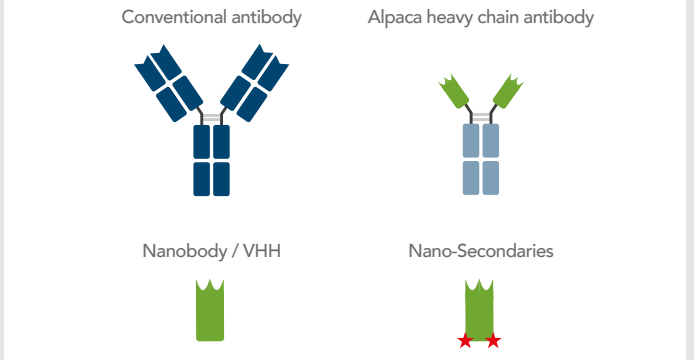


Figure 3. Nanobodies are the antigen binding domains or variable heavy domain of heavy chain antibodies (VHHs) from alpacas, llamas, or camels and are the smallest known antibodies (~12-15 kDa vs ~150 kDa of conventional IgG antibodies). Nano-Secondaries are Nanobodies conjugated to fluorescent dyes binding to conventional antibodies.

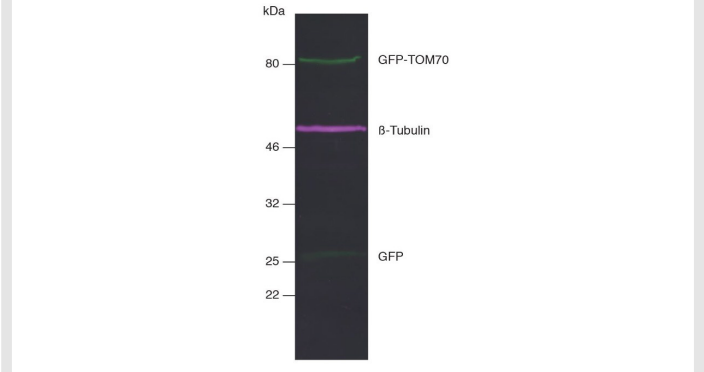


Figure 4. Multiplex fluorescent Western blot of GFP-TOM70, β-Tubulin, and GFP in HEK293T cell lysate. Western blot membrane was simultaneously incubated with primary antibodies and Nano-Secondaries. Green: rabbit anti-GFP (ChromoTek PABG1) + alpaca anti-rabbit IgG VHH Alexa Fluor® 488. Magenta: mouse anti-β-Tubulin + alpaca anti-mouse IgG2b VHH Alexa Fluor® 647.

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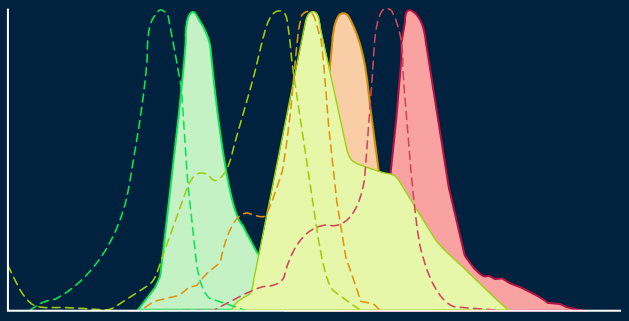
Nano-Secondaries for multiplex fluorescent western blots



Product Name	Conjugate	Species Reactivity	Catalogue No.
Nano-Secondary® alpaca anti-human IgG, recombinant VHH [CTK0101, CTK0102]	Alexa Fluor® 488	Rabbit, Human, Macaque	srbAF488-1
	Alexa Fluor® 568	Rabbit, Human, Macaque	srbAF568-1
	Alexa Fluor® 647	Rabbit, Human, Macaque	srbAF647-1
Nano-Secondary® alpaca anti-mouse IgG1, recombinant VHH [CTK0103, CTK0104]	Alexa Fluor® 488	Mouse	sms1AF488-1
	Alexa Fluor® 568	Mouse	sms1AF568-1
	Alexa Fluor® 647	Mouse	sms1AF647-1
Nano-Secondary® alpaca anti-mouse IgG2b, recombinant VHH [CTK0105, CTK0106]	Alexa Fluor® 488	Mouse	sms2bAF488-1
	Alexa Fluor® 568	Mouse	sms2bAF568-1
	Alexa Fluor® 647	Mouse	sms2bAF647-1
Nano-Secondary® alpaca anti-mouse IgG3, recombinant VHH [CTK0107]	Alexa Fluor® 647	Mouse	sms3AF647-1
Nano-Secondary® alpaca anti-rabbit IgG, recombinant VHH [CTK0101, CTK0102]	Alexa Fluor® 488	Rabbit, Human, Macaque	srbAF488-1
	Alexa Fluor® 568	Rabbit, Human, Macaque	srbAF568-1
	Alexa Fluor® 647	Rabbit, Human, Macaque	srbAF647-1

Tag/Control Antibodies

Running western blots in the presence of appropriate controls is key to ensure correct interpretation of experimental results and for generating publication worthy data. Proteintech offers a wide range of tag/control antibodies as indicated in the table below.

Loading control antibodies					Isotype control antibodies	Tag antibodies
Whole cell/cytoplasmic proteins	Mitochondrial proteins	Nuclear proteins	Plasma membrane proteins	Serum samples		
Alpha actin Beta actin Alpha tubulin Beta tubulin Gamma tubulin GAPDH Vinculin	COX1V COX412 HSP60 VDAC1/Porin	Lamin A/C Lamin B1 PCNA Histone H1 Histone H3 TBP	ATP1A1	Transferrin	Mouse IgG1 Mouse IgG2a Mouse IgG2b Rabbit IgG	Myc Flag His, 6X His GFP GST HA MBP V5 mFC S1

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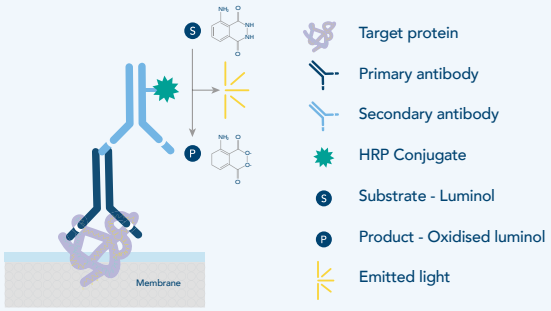
Chemiluminescent Substrates

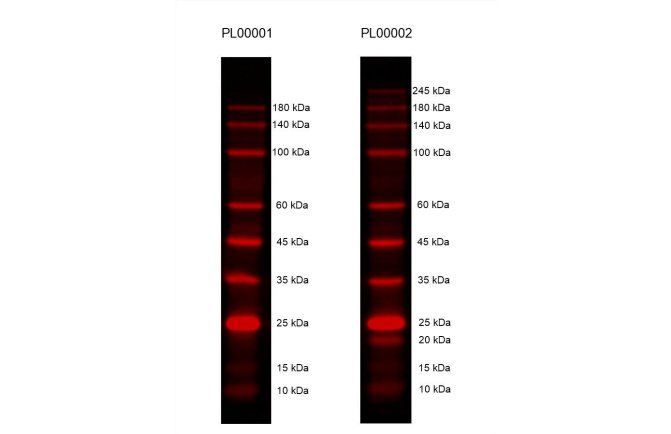
Proteintech's SignalBright Chemiluminescent Substrate range of SignalBright Pro (PK10011), SignalBright Plus (PK10012), and SignalBright Max (PK10013) is perfect for the detection of femtogram levels of protein. We offer chemiluminescent substrates with different levels of sensitivity and signal duration. See the table of comparisons below to select which substrate is right for you.

Increasing Sensitivity →

	SignalBright Pro	SignalBright Plus	SignalBright Max
Sensitivity	Mid/high to high femtogram	Mid to high femtogram	Low to mid femtogram
Relative sensitivity to SignalBright Pro	1x	4x	10x
Stable signal duration	>5 hours	>3 hours	>2 hours
Primary antibody concentration	0.02 - 1 μg/ml	0.02 - 0.5 μg/ml	0.01 - 02 μg/ml
Secondary antibody concentration	4 - 20 ng/ml	2 - 20 ng/ml	1 - 10 ng/ml

Detect low abundance proteins or precious samples with SignalBright Chemiluminescent Substrates





Protein Ladders

- Standard prestained protein ladder (PL00001)
- Broad range prestained protein ladder (PL00002)

Figure 5. 4ul protein ladders were loaded in the gel with concentration 8%-18%. After Electrophoresis, the protein on the gel were transformed into the PVDF membrane. This image was taken by an iBright™ imager.

For more information visit **ptglab.com**