Western Blotting

Western blotting (WB) is a fundamental technique that has helped scientists understand important signaling pathways, protein associations, protein expression in various cell lines and tissues, and effects of drugs or treatments on protein levels. BioLegend provides helpful tools for WB use, from molecular weight protein ladders and substrate buffers to highly specific antibodies. We are committed to providing every essential tool necessary to obtain distinct and easy–to–interpret bands in your blots.

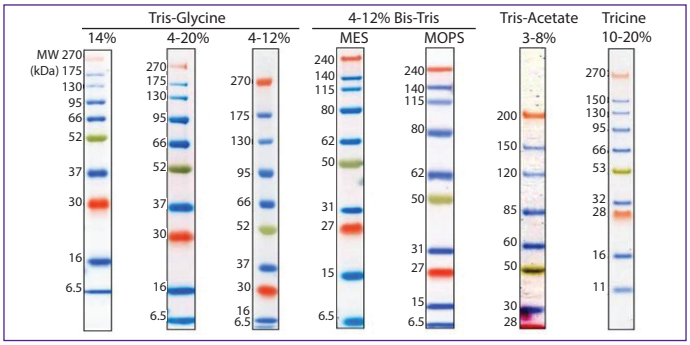
To find more western blotting antibodies and reagents, visit:
biolegend.com/en-us/western-blot

Accurate Protein Ladders

The Prime–Step™ Prestained Broad Range Protein Ladder is a three–color protein standard with 10 prestained chromophore–conjugated recombinant proteins covering a wide range of molecular weights, from 6.5 to 270 kDa. It is ideal for monitoring protein separation during SDS–PAGE, confirming WB transfer on membranes, and estimating protein sizes.

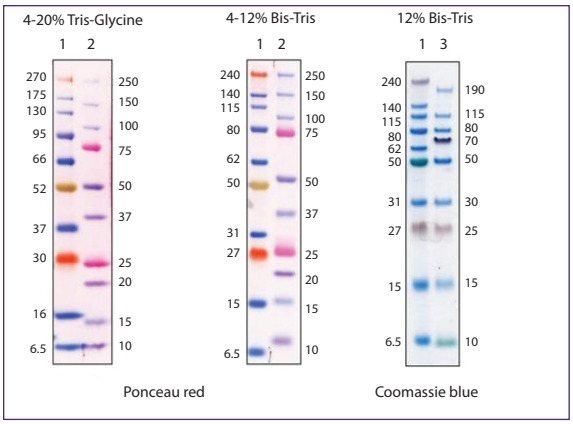
Description	Size	Cat. No.
Prime-Step™ Prestained Broad Range Protein Ladder	50 µL | 500 µL	773301 | 773302

Clear separation and sharp bands in different SDS-PAGE gel and buffer systems



3 µL Prime–Step™ Prestained Broad Range Protein Ladder was resolved by electrophoresis in different gel and running buffer systems, followed by transfer to nitrocellulose membrane. The molecular weight (MW) (kDa) of each protein was determined by calibration against unstained protein standards. Note: All MWs listed are approximate values.

Comparable MW detection with competitors' protein ladders



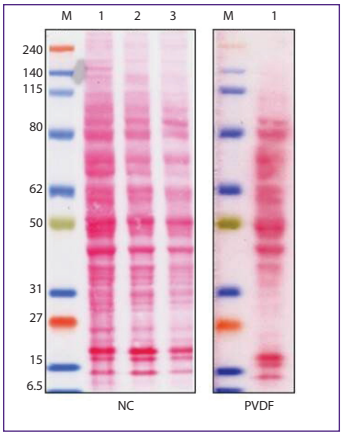
3 µL Prime–Step™ Prestained Broad Range Protein Ladder was resolved by electrophoresis in different gel running systems as indicated. Proteins were either stained with Coomassie blue, or transferred to nitrocellulose membrane followed by Ponceau red staining. Note: All MWs listed are approximate values.

Lane 1: Prime–Step™ Prestained Broad Range Protein Ladder

Lane 2: Competitor 1 protein ladder

Lane 3: Competitor 2 protein ladder

Excellent transfer efficiency for WB



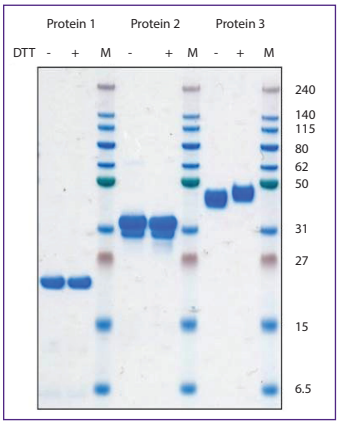
3 µL Prime–Step™ Prestained Broad Range Protein Ladder (lane M) and total cell lysates (15 µg total protein) was resolved by electrophoresis in 4–12% Bis–Trisgel (MOPS running buffer). Then protein ladders and total cell lysates were transferred to nitrocellulose (NC) and Polyvinylidene difluoride (PVDF) membrane followed by Ponceau red staining. Note: All MWs listed are approximate values.

Lane 1: HeLa

Lane 2: Jurkat

Lane 3: NIH3T3 cells

Suitable for size detection during protein purification



3 µL Prime–Step™ Prestained Broad Range Protein Ladder (lane M) and 2 µg recombinant proteins 1, 2, 3, with different molecular weights, was resolved by electrophoresis in 12% Bis–Trisgel (MES running buffer) followed by Coomassie blue staining. Note: All MWs listed are approximate values.

BioLegend®

Western–Ready™ Buffers

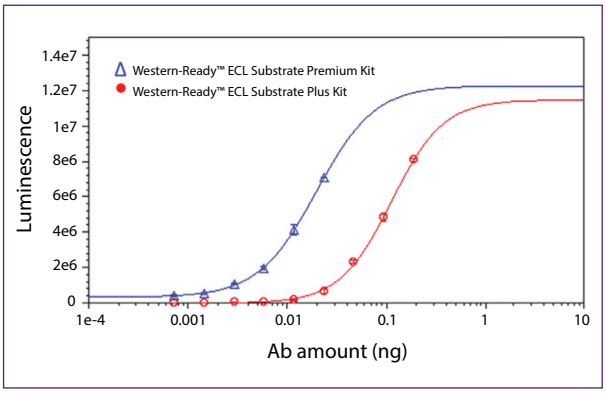
Try our complete set of Western–Ready™ reagents for every step of your workflow, including buffers for protein extraction, gel loading and running, and detection.

Features of our Western–Ready™ Buffers:

- Quality tested in–house
- Performance is guaranteed 100%

Description	Use	Size	Cat. No.
Western-Ready™ ECL Substrate Plus Kit	The ECL Substrate Plus Kit is useful for detecting picogram amounts of antigen.	20 mL 200 mL 500 mL	426315 426316 426317
Western-Ready™ ECL Substrate Premium Kit	The ECL Substrate Premium Kit detects femtogram amounts of antigen.	20 mL 200 mL	426318 426319
Western-Ready™ TBS Tween-20 Buffer (20X)	Buffer for blocking, washing, and antibody dilution. Formulated to reduce non-specific binding.	1 L	426309
Western-Ready™ Rapid Protein Extraction Buffer	Stringent lysis buffer to extract proteins from all subcellular compartments.	100 mL	426305
Western-Ready™ MES SDS-PAGE Running Buffer (10X)	Running buffer for resolving small-to-medium-sized proteins on Bis-Tris SDS page gels.	1 L	426307
Western-Ready™ Protein Sample Loading Buffer (5X)	Used for the preparation of samples for non-reducing and reducing SDS-PAGE.	20 mL	426311

Western-Ready™ ECL Substrate Kits



ELISA analysis using Western–Ready™ ECL Substrate Plus and Premium Kits. The indicated amounts of purified anti–SSRP1 antibody (Cat. No. 609702) were captured by anti–mouse Fc–coated plates for 1 hour at room temperature. Bound antibody was detected with HRP goat anti–mouse IgG antibody (Cat. No. 405306) using either Western–Ready™ ECL Substrate Premium Kit (Cat. No. 426319) (blue triangles) or Western–Ready™ ECL Substrate Plus Kit (red circles). Shown values represent the average signal of three technical replicates after background subtraction.

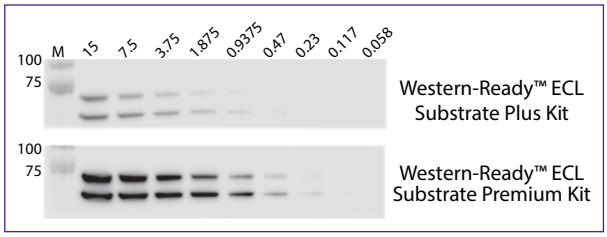
Western–Ready™ ECL Substrate Kits

Sensitive Substrates

Our Western–Ready™ ECL Substrate Kits are a non–radioactive enhanced luminol–based chemiluminescent substrate (ECL) for the detection of horseradish peroxidase (HRP) activity from WB reagents and ELISA immunoassays. Our kits provide sensitive detection and sustained signal for up to 4 hours. Our ECL Plus Kit is ideal for detecting low–to mid–picogram levels of antigen, while the Premium Kit provides more sensitive detection of high–femtogram levels of protein.

Key Features:

- Luminol–based detection system
- Sensitive detection
- Available in small 20 mL size for sampling
- Multiple kits with a range of sensitivities



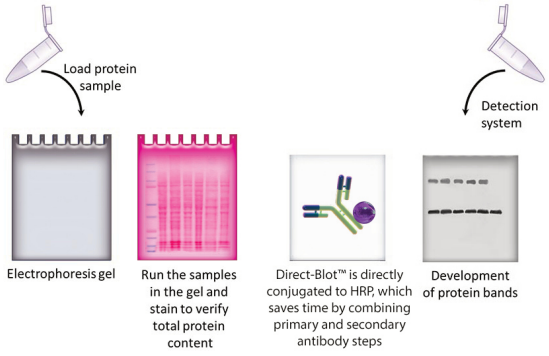
Western blot analysis using Western–Ready™ ECL Substrate Plus and Premium Kits. The indicated amounts of whole cell extract from HeLa cells were resolved by 4–12% Bis–Trisgel electrophoresis, transferred to a nitrocellulose membrane, and probed with 0.1 µg/mL (1:5000 dilution) of purified anti–Lamin A/C antibody (Cat. No. 600002) overnight at 4°C. Proteins were visualized using HRP goat anti–mouse IgG antibody (Cat. No. 405306) at a 1:3000 dilution, followed by incubation with Western–Ready™ ECL Substrate Plus Kit or Western–Ready™ ECL Substrate Premium Kit.

REFERENCIA	DESCRIPCIÓN	TAMAÑO	PRECIO VENTA
773301	Prime–Step™ Prestained Broad Range Protein Ladder	50 µL	34,00 €
773302	Prime–Step™ Prestained Broad Range Protein Ladder	500 µL	118,00 €
426315	Western–Ready™ ECL Substrate Plus Kit	20 mL	56,00 €
426316	Western–Ready™ ECL Substrate Plus Kit	200 mL	168,00 €
426317	Western–Ready™ ECL Substrate Plus Kit	500 mL	280,00 €
426318	Western–Ready™ ECL Substrate Premium Kit	20 mL	84,00 €
426319	Western–Ready™ ECL Substrate Premium Kit	200 mL	560,00 €
426305	Western–Ready™ Rapid Protein Extraction Buffer	100 mL	135,00 €
426309	Western–Ready™ TBS Tween–20 Buffer (20X)	1 L	135,00 €
426311	Western–Ready™ Protein Sample Loading Buffer (5X)	20 mL	45,00 €
426307	Western–Ready™ MES SDS–PAGE Running Buffer (10X)	1 L	73,00 €

Direct–Blot™ HRP Primary Antibodies

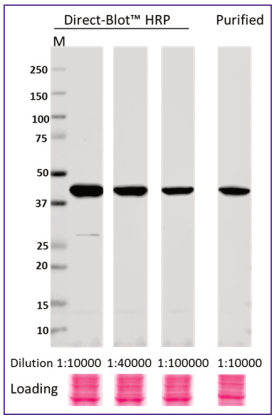
Antibodies Crafted for Specificity

When performing a WB, it is common to use a secondary antibody to detect your proteins of interest. However, unless the signal needs to be amplified, there is no advantage to using a two–step process. We provide Direct–Blot™ antibodies, horseradish peroxidase (HRP) directly conjugated primary antibodies, to eliminate the need for a secondary antibody and get you to your results faster. Our HRP directly conjugated antibodies are carefully developed and tested to ensure high sensitivity.



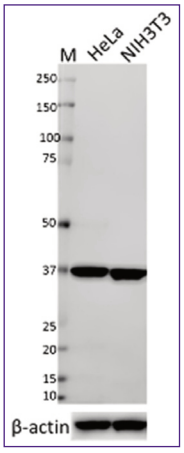
Featured Direct–Blot™ Data

Direct-Blot™ HRP anti-β-actin Antibody



Whole cell lysates (15 µg protein) from HeLa was resolved by electrophoresis (4–20% Tris–glycinegel), transferred to nitrocellulose, and probed with 1:10,000, 1:40,000, and 1:100,000 dilutions of Direct–Blot™ HRP anti–β–actin Antibody, clone W16197A, or a 1:10,000 dilution of purified anti–β–actin Antibody, clone W16197A (upper). Proteins were visualized using a 1:3000 diluted HRP anti rat–IgG secondary antibody for purified anti–β–actin Antibody and chemiluminescence detection. Ponceau S staining was used as loading control (lower). Lane M: MW ladder.

Direct-Blot™ HRP anti-GAPDH Antibody



Whole cell lysates (15 µg protein) from HeLa and NIH3T3 cells were resolved by electrophoresis (4–12% Bis–Tris gel), transferred to nitrocellulose, and probed with 1:4000 Direct–Blot™ HRP anti–GAPDH antibody, clone W17079A (upper). 1:2000 dilution of Direct–Blot™ HRP anti–β–actin antibody (Cat. No. 643807) was used as a loading control (lower). Proteins were visualized using chemiluminescence detection. Lane M: MW ladder.

Find more Direct-Blot™ antibodies at:
biolegend.com/en-us/direct-blot

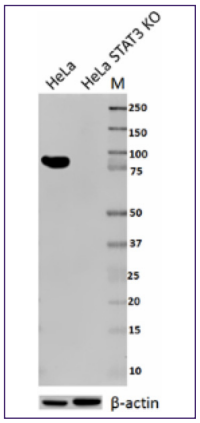
Knockout and Knockdown Validated Antibodies

The reproducibility of published research has emerged as an urgent topic in today's scientific community. From funding agencies to researchers to manufacturers and publishers, it is critical for all of these groups to align themselves to ensure that research is done with rigor and is reproducible. We guarantee antibody specificity of our antibody products. As knocking out the target protein is one of the most trusted antibody validation processes, we validate many of our cell biology antibodies by KO (knockout) and KD (knockdown) systems.

Learn more at:
biolegend.com/en-us/kokd-validation

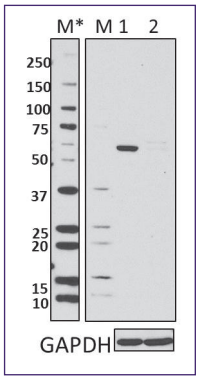
Featured KO/KD Data

STAT3



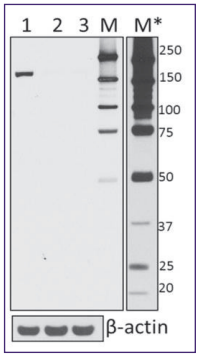
Total lysates (15 µg protein) from HeLa and HeLa STAT3 CRISPR/Cas9 knockout (KO) cells were resolved by electrophoresis (4–20% Tris–glycinegel), transferred to nitrocellulose, and probed with 1:500 (1 µg/ml) purified anti–STAT3 antibody, clone 4G4B45 (upper). Proteins were visualized using chemiluminescence detection by incubation with HRP goat anti–mouse–IgG secondary antibody (Cat. No. 405306). Direct–Blot™ HRP anti–β–actin Antibody (Cat. No. 643807) was used as a loading control (lower). Lane M is the molecular weight ladder.

HDAC2



Total lysates from 293T (lane 1) and 293T/HDAC2 CRISPR/Cas9 knockdown (KD) cells (lane 2) were probed with purified anti–HDAC2 antibody (clone 13G8C67). Anti–GAPDH (poly6314) antibody was used as a loading control. Lane M: MW ladder, M* indicates longer exposure.

TSC2



Total lysates from HeLa (Lane 1), 5 nM (Lane 2), and 20 nM (Lane 3) TSC2 siRNA treated HeLa cells were probed with purified anti–TSC2 antibody (clone W16104A). Direct–Blot™ HRP anti–β–actin Antibody was used as a loading control. M is MW ladder. M* is higher exposure of the ladder.

Research Multiple Parts of a Signaling Pathway

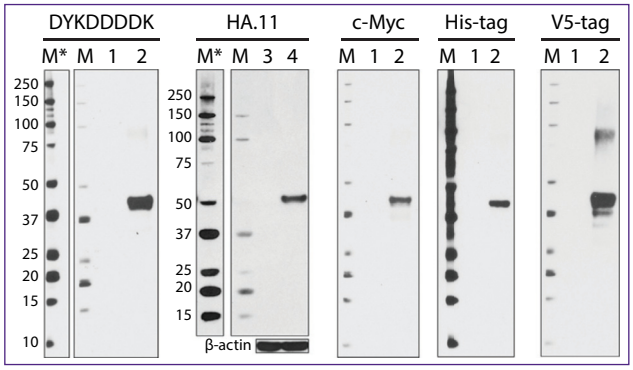
Antibody sampler kits provide a convenient and economical resource to test multiple antibodies. Our kits target several cell function and structure proteins, related detection methods (such as epitope tags), or proteins connected through signaling pathways.

PhosphoPair antibody sets bundle a pair of antibodies together for cost–savings and convenience. One antibody is specific for a phosphorylated form of a protein, while the other detects total or unphosphorylated levels of the protein. Our PhosphoPair antibody sets are quality tested in western blotting to ensure high quality results in each experiment.

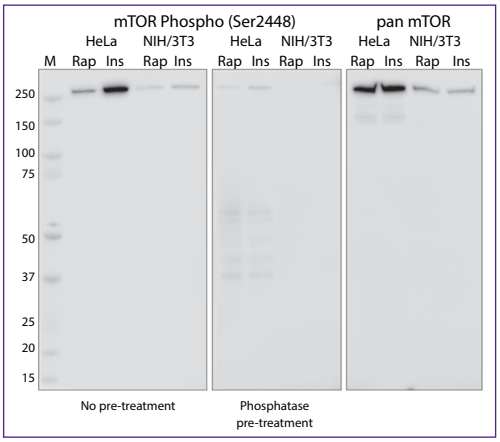
Advantages of our kits and PhosphoPair sets:

- Antibodies are quality tested in–house for western blotting and validated for multiple applications
- Sufficient antibody is provided in each kit or set to run multiple assays
- Performance is guaranteed 100%

For more information, visit:
biolegend.com/en-us/sampler-kits



50 ng recombinant human IFN–γ protein (Lane 1, negative control), Posi–Tag Epitope Tag Protein (Lane 2, positive control), 15 µg each of total protein from CHO cells (Lane 3), and CHO transfected with HA tagged protein (lane 4) were probed with anti–DYKDDDDK (clone L5), anti–HA.11 (clone 16B12), anti–C–Myc (clone 9E10), anti–His (clone J099B12), and anti–V5 antibody (clone 7/4). Direct–Blot™ HRP anti–β–actin Antibody (clone 2F1–1) was used as a loading control. Lane M: MW ladder. Lane M*: long exposure MW ladder.



Western blot analysis using whole cell extracts (15 µg protein) from serum starved HeLa and NIH/3T3 cells treated with 10 µM rapamycin (Rap) for 2 hours or with 150 nM insulin (Ins) for 6 minutes were resolved by 4–12% Bis–Tris gel electrophoresis, transferred to a nitrocellulose membrane, and probed with 0.5 µg/mL (1:1000 dilution) of purified anti–mTOR Phospho (Ser2448), clone A17024A (Cat No. 610301), for 2 hours at room temperature. To confirm phospho–specificity, a duplicate membrane was pre–treated with lambda protein phosphatase prior to incubation. Proteins were visualized by chemiluminescence detection using HRP goat anti–mouse–IgG (Cat. No. 405306) at a 1:3000 dilution. Equal mTOR loading was confirmed by probing membranes with an anti–pan mTOR antibody (Cat. No. 659202) at 1.0 µg/mL (1:500 dilution). Lane M: Molecular Weight Marker.

Antibody Sampler Kits

Description	Clones	Specificities	Cat. No. (1 kit)
E.coli RNA Polymerase Antibody Sampler Kit	NT73, NT63, 4RA2, 8RB13	RNA Polymerase β prime, β, α	699907
Epitope Tag Small Motif Antibody Sampler Kit	L5, 9E10, 16B12, 7/4, J099B12	DYKDDDDK tag, Myc tag, HA tag, V5 tag, His tag	699903
Epitope Tag Big Motif Antibody Sampler Kit	P1A12, 1GFP63, 8C5.5, 4H12A59	GST tag, GFP tag, mCherry tag, Thioredoxin tag	699902
IRF Antibody Sampler Kit	13H3A44, 13B2A38, 12A4A35, IRF4.3E4, 11F4A09, 14B2C16, 12G9A36, 7G11A45, 5A3A39	IRF 1 - 9	699906
α-Synuclein Antibody Sampler Kit	A15119B, P-syn/81A, A15115A, A15127A, A15126D	α-Synuclein Phospho (Tyr39), α-Synuclein Phospho (Ser129), α-Synuclein (80-96), α-Synuclein (C-Terminal Truncated x-122), α-Synuclein (117-122)	899903
Tau Antibody Sampler Kit	A15091A, M7004D06, A16103A, A16097F	Tau Phospho (Ser262), Tau Phospho (Thr181), Tau (1-223), Tau (368-441)	899902
Glial Cell Marker Antibody Sampler Kit	S16007D, 8E12.D9, SMI 24, SMI 91, P82H9	P2RY12, CX3CR1, GFAP, Myelin CNPase, Myelin Basic Protein	899904

PhosphoPair Sets

Description	Clones	Specificities	Cat. No.
PhosphoPair STAT1 Antibody Set	10C4B40, A15158B	STAT1 (Ser727)	699955
PhosphoPair STAT3 Antibody Set	4G4B45, 13A3-1	STAT3 (Tyr705)	699952
PhosphoPair PLK-1 Antibody Set	3F8, 2A3	PLK-1 (Thr210)	699956

Description	Clones	Specificities	Cat. No.
PhosphoPair ERK1/2 Antibody Set	6B8B69, W15133B	ERK1/2 (Thr202) ERK1/2(Tyr204)	699951
PhosphoPair mTOR Antibody Set	6H9B10, A17024A	mTOR(Ser2448)	699953
PhosphoPair Lck Antibody Set	LCK-01, A18002D	Lck(Tyr394)	699954

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