Elabscience®

Visualizing the Proteome, Validating Discovery

Integrated Solutions for High-Confidence Western Blot Analysis



Elabscience Bionovation Inc.

Elabscience® stands at the forefront of biotechnological innovation, delivering high-fidelity cellular detection reagents, precision research tools, and integrated bioanalytical solutions for advanced life science research.

Its portfolio spans protein and antibody reagents, flow cytometry antibody systems, functional cell assay kits, metabolic detection platforms, and ELISA technologies, enabling precise interrogation of complex biological systems.

Anchored in ISO 9001-certified quality systems and aligned with EU CE regulatory compliance, Elabscience® delivers globally validated products across 150+ countries and regions, supporting reproducible, high-fidelity research in immunology, cell biology, and translational biomedical sciences.



ELISA Kits Supplier to Watch in 2019 CiteAb



Watch in 2021 CiteAb



ISO9001:2015 Quality Management System Certification

130,000 ft² +

State-of-the-Art Research & Office Facilities

160,000 ft² +

Purpose-Built Research Laboratories

28,500 +

SCI Citations (as of December 2025)

103,300 +

Cumulative Impact Factor (as of December 2025)

Elabscience®

Contents

Cornerstone of Protein Research ---- 01

01 Preparing High-Quality Protein Samples

High-Yield Total Protein Extraction ---- 02
Sonication ---- 02
Protein Quantification ---- 02
Protein Denaturation ---- 03

02 Gel Electrophoresis

Gel Concentration Selection ---- 03
Select the Appropriate Protein Marker ---- 03
Electrophoresis Buffer ---- 04
Optional Gel Staining ---- 04

03 Protein Transfer

Optimal Transfer Method ---- 05
Membrane Selection ---- 05
Transfer Conditions ---- 05
Optional Ponceau S Staining ---- 06

04 Blocking

Blocking Procedure ---- 07

05 Antibody Incubation

Antibody Incubation Workflow ---- 08

06 Signal Detection and Imaging

Workflow ---- 08

07 Common Western Blot Issues and Solutions 09

08 Control Antibodies 10

09 Tag Antibodies 11

10 Citations 12

Elabscience®

Cornerstone of Protein Research

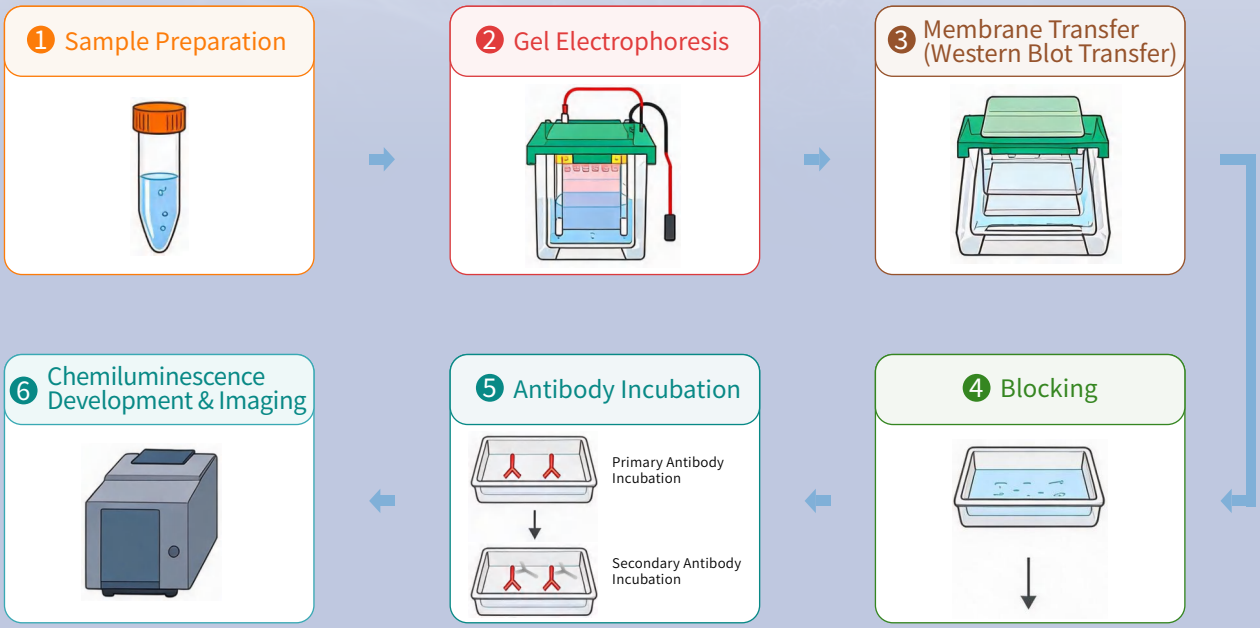
Western blot (WB) remains the gold standard for protein identification and molecular weight determination, harnessing highly specific antigen–antibody interactions to deliver sensitive and reliable protein detection across diverse research applications.

Principle

Following separation by SDS-polyacrylamide gel electrophoresis (SDS-PAGE), proteins are electrophoretically transferred onto a membrane and immobilized for immunodetection. After blocking nonspecific binding sites, target proteins are sequentially recognized by specific antibodies and visualized using colorimetric, chemiluminescent, or radioactive detection, enabling high-sensitivity protein analysis with exceptional specificity.

Experimental Workflow

A standard western blot workflow comprises sample preparation, SDS-PAGE separation, protein transfer, membrane blocking, antibody incubation, and signal development/imaging.



01 Preparing High-Quality Protein Samples

High-Yield Total Protein Extraction

Commonly used RIPA lysis buffers provide efficient total protein extraction; the optimal lysis formulation should be selected according to the biochemical properties and localization of target proteins.



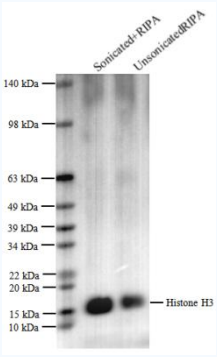
RIPA Lysis Buffer (Strong) [E-BC-R327]



Total Protein Extraction Assay Kit [E-BC-E002]

Sonication

Following chemical or physical lysis of cell/tissue samples, optional sonication can be applied to reduce sample background and enhance target protein detectability.
Sonication conditions should be adjusted according to sample volume and lysis efficiency. Optimal treatment is achieved when the sample becomes homogeneous with no apparent viscosity.



Target Nuclear protein Histone H3

Molecular Weight 15 kDa

Protein Extraction Conditions Experimental group: Unsonicated + RIPA
Control group: Sonicated + RIPA

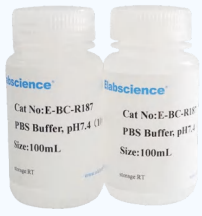
Result Sonication-assisted protein extraction produced a stronger target protein signal, indicating enhanced extraction efficiency and improved target protein detectability.

Protein Quantification

Protein concentration is determined by generating a standard curve using reference proteins and calculating sample concentrations based on measured absorbance values.



BCA Protein Colorimetric Assay Kit [E-BC-K318]



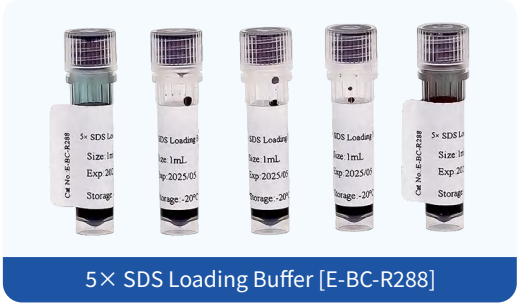
PBS Buffer, pH7.4 (10×) [E-BC-R187]

Protein Denaturation

Loading buffer treatment and heat-induced denaturation eliminate protein conformational complexity, enabling uniform unfolding and optimized electrophoretic separation.



2× SDS Loading Buffer [E-IR-R329]



5× SDS Loading Buffer [E-BC-R288]

02 Gel Electrophoresis

SDS-PAGE, a cornerstone of protein separation, integrates stacking and resolving gels to achieve precise molecular weight-based fractionation. The stacking gel concentrates protein samples to enhance band sharpness and consistency, while the resolving gel enables effective separation according to protein size.

Gel Concentration Selection

Optimized acrylamide concentration selection is critical for achieving optimal protein resolution. The stacking gel is typically prepared at 5%, while the resolving gel concentration is selected according to the molecular weight range of target proteins to ensure effective separation.

Target Protein Molecular Weight Range (kDa)	Resolving Gel Concentration
120<X< 300	6%
80<X<120	8%
30 <X< 80	10%
15<X< 30	12%
10 <X< 15	15%

Note: X represents the molecular weight of the target protein.

Simplify gel preparation with Elabscience® SDS-PAGE Gel Preparation Kits, featuring a complete reagent system for efficient, consistent, and reproducible SDS-PAGE gel casting.



SDS-PAGE Gel kit [E-IR-R305]



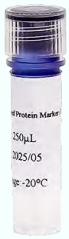
PAGE Gel Quick Preparation Kit[E-IR-R338]

Select the Appropriate Protein Marker

Selecting a protein marker that matches the molecular weight range of the target protein enables accurate band localization and reliable molecular weight estimation.



Pre-stained Protein Marker (10~180 kDa) [E-BC-R273]



Pre-stained Protein Marker (10~250 kDa) [E-IR-R331]

Electrophoresis Buffer

Pre-chilled electrophoresis buffer minimizes heat generation during electrophoresis, reducing band diffusion and enhancing protein separation with improved resolution.



Electrophoresis Buffer (10×) [E-BC-R331]



MOPS Running Buffer (20×) [E-IR-R339]



MES Running Buffer (20×) [E-IR-R340]



SDS-PAGE Electrophoresis Buffer with Tris-Gly (10×) [E-IR-R341]

Optional Gel Staining

Following electrophoresis, Coomassie Brilliant Blue staining provides a rapid assessment of protein separation quality, abundance, band migration consistency, and lane integrity. This step may be omitted when proceeding directly to protein transfer.



Fast Coomassie Blue Staining Solution (10×) [E-IR-R129]

03 Protein Transfer

Transfer is a critical step in western blotting, where electrophoretically separated proteins are driven from the SDS-PAGE gel onto a solid membrane substrate, such as PVDF, under an applied electric field for subsequent immunodetection.

Optimal Transfer Method

Semi-dry transfer: Requires minimal transfer buffer and enables rapid protein transfer, but offers relatively lower stability; commonly suitable for small molecular weight proteins.
Wet transfer: Provides enhanced transfer stability with higher buffer consumption and longer transfer duration, making it suitable for a broad range of protein sizes.



Transmembrane Buffer (10×)(E-BC-R333)



Western Blot Rapid Transfer Buffer(10×)(E-IR-R342)

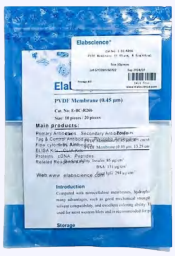
Membrane Selection

Membrane selection should be optimized according to the molecular weight of the target protein to ensure efficient transfer and reliable detection.

Target protein >25 kDa: Recommended 0.45 μm NC membrane or PVDF membrane
Target protein <25 kDa: Recommended 0.22 μm NC membrane or PVDF membrane



PVDF Membrane (0.22 μm) [E-BC-R329]



PVDF Membrane (0.45 μm) [E-BC-R266]

Transfer Conditions

Transfer parameters should be tailored to target protein size, with optimized methanol concentration and transfer conditions selected to achieve efficient and consistent protein migration onto the membrane.

Target Protein Molecular Weight (kDa)	Transfer Buffer (Methanol Concentration)	Recommended Transfer Method	Recommended Transfer Conditions
X> 150	5-10% or lower	Wet Transfer	Constant current 200-300 mA, transfer for >2 h
20 < X< 150	10-15%	Wet / Semi-dry Transfer	Constant current 200-300 mA, transfer for 1-2 h; or constant voltage 10-25 V, transfer for 10-30 min
X< 20	15-20%	Wet Transfer	Constant current 150-300 mA, transfer for 0.5-1.5 h

Note: X represents the molecular weight of the target protein.

Optional Ponceau S Staining

Following protein transfer, Ponceau S staining enables rapid visualization and assessment of transfer efficiency. Through reversible binding to membrane-bound proteins, Ponceau S provides a convenient quality check while allowing easy removal without interfering with subsequent antibody incubation.



Ponceau S Solution[E-IR-R127]

Transfer Workflow

Transfer Preparation

Prepare PVDF membrane and filter papers according to gel size. Activate the PVDF membrane by methanol treatment for 1–2 min, followed by equilibration in 1× transfer buffer. Pre-soak filter papers and fiber pads in transfer buffer. Transfer buffer should be pre-cooled at −20°C for at least 2 h before use.

Transfer Assembly

Assemble the transfer sandwich in the sequence of cathode (black plate) → fiber pad → filter paper → gel → membrane → filter paper → fiber pad → anode (white plate). Remove air bubbles thoroughly and secure the transfer cassette before placing it into the wet transfer system.

Transfer Operation

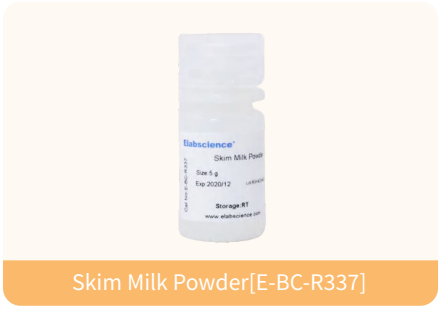
Add transfer buffer and apply optimized transfer conditions according to membrane type and target protein molecular weight.

Transfer Validation

Stain the membrane with Ponceau S for 5–10 min to evaluate transfer efficiency. Clear protein marker bands indicate successful protein transfer.

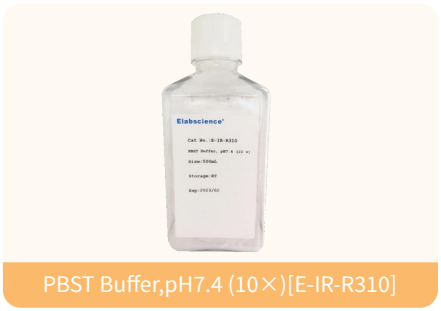
04 Blocking

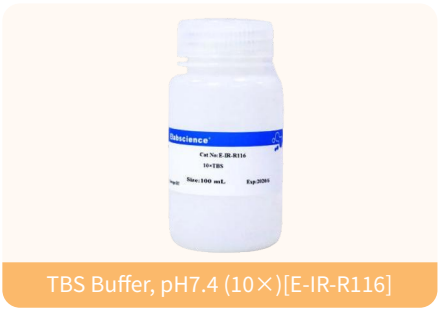
Following protein transfer, residual unoccupied binding sites on the membrane are masked through blocking treatment, minimizing nonspecific antibody interactions and reducing background signals for improved detection specificity.

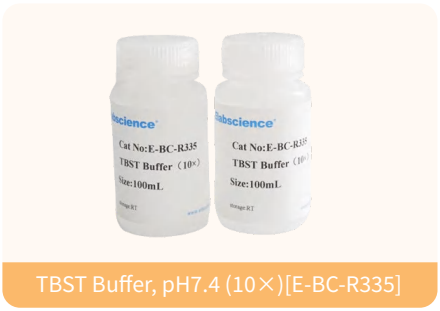

Skim Milk Powder[E-BC-R337]


Blocking Powder[E-IR-R108]


Western Blot Fast Blocking Solution[E-IR-R343]


PBST Buffer,pH7.4 (10×)[E-IR-R310]


TBS Buffer, pH7.4 (10×)[E-IR-R116]



TBST Buffer, pH7.4 (10×)[E-BC-R335]

Blocking Procedure

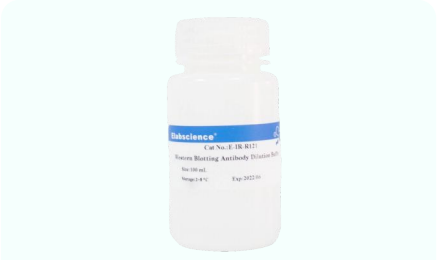
- Membrane Washing: Rinse the membrane with TBST for 1 min.
- Blocking Incubation: Incubate the membrane with blocking solution at room temperature for 1–2 h or at 4°C overnight to minimize nonspecific antibody binding and reduce background signals.

05 Antibody Incubation

Primary antibody specifically recognizes the target protein, while the secondary antibody binds the primary antibody and enables signal detection through fluorescent or enzymatic labels. Select a secondary antibody compatible with the host species of the primary antibody (e.g., rabbit primary antibody paired with anti-rabbit secondary antibody).

Choose an appropriate antibody diluent according to antibody characteristics to optimize detection sensitivity and specificity.


Western Antibody Dilution Buffer (Block Quickly)[E-IR-R125]


Western Blotting Antibody Dilution Buffer [E-IR-R121]



Western Blot Stripping Buffer (Neutral) [E-IR-R102]

Antibody Incubation Workflow

- **Primary Antibody Incubation**

Remove blocking solution and wash the membrane with TBST. Incubate with diluted primary antibody at room temperature for 1–2 h or at 4°C overnight.

- **Primary Antibody Washing**

Remove excess primary antibody and wash the membrane with TBST three times for 5 min each.

- **Secondary Antibody Incubation**

Incubate the membrane with diluted secondary antibody at room temperature for 1 h.

- **Secondary Antibody Washing**

Remove excess secondary antibody and wash the membrane with TBST three times for 5 min each.

06 Signal Detection and Imaging

Western blot detection commonly utilizes HRP-conjugated secondary antibodies combined with enhanced chemiluminescence (ECL) substrates to generate and visualize target protein signals.



Excellent Chemiluminescent Substrate (ECL) Detection Kit[E-IR-R307]



Super Excellent Chemiluminescent Substrate (ECL) Detection Kit [E-IR-R308]

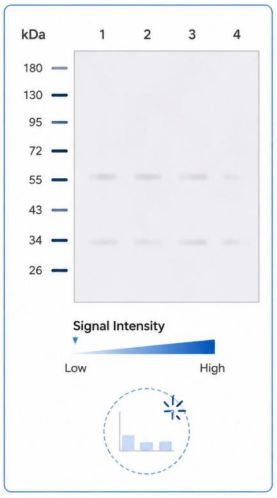
Workflow

- Remove the washed membrane and gently blot away residual TBST using filter paper.
- Evenly apply the ECL substrate to the membrane and incubate for 1–2 min.
- Place the membrane into the imaging system, optimize exposure time according to signal intensity, and capture the protein band image.

07 Common Western Blot Issues and Solutions

1 Weak or No Signal

Faint or no detectable bands on the membrane.



Possible Causes

- Low target protein expression
- Antibody incompatibility
- Insufficient protein transfer

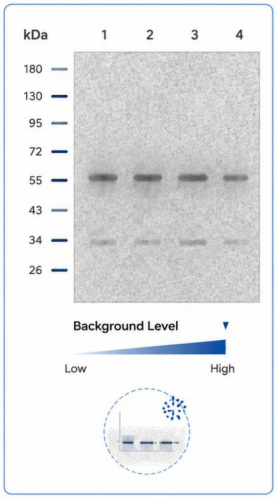
Optimization

- Optimize sample loading
- Validate antibody specificity
- Improve transfer efficiency

TIP
Ensure sample integrity and confirm that each step of the experiment is optimized for efficient protein detection.

2 High Background

Excessive non-specific signal or uniform background across the membrane.



Possible Causes

- Incomplete blocking
- Excess antibody concentration
- Insufficient washing

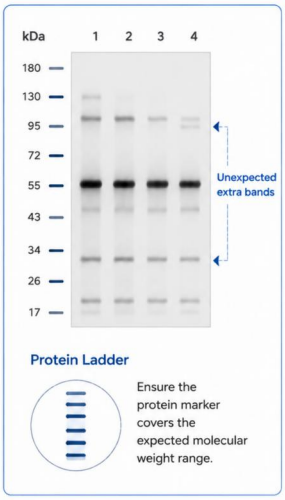
Optimization

- Optimize blocking conditions
- Adjust antibody dilution
- Improve washing efficiency

TIP
Reducing non-specific binding and improving washing conditions are key to achieving a clean, low-background result.

3 Unexpected Bands

Additional bands appear at unexpected molecular weights.



Possible Causes

- Protein degradation
- Post-translational modifications (glycosylation, phosphorylation, etc.)
- Non-specific antibody recognition

Optimization

- Add protease inhibitors during sample preparation
- Verify whether the target protein undergoes post-translational modifications
- Confirm antibody specificity (use validated antibodies)

TIP
Unexpected bands can result from sample issues or non-specific interactions. Systematically verify sample quality and antibody specificity to ensure reliable results.

4 Poor Band Resolution

Blurry or smeared bands with poor separation.



Possible Causes

- Excess sample loading
- Improper gel selection
- Incomplete transfer

Optimization

- Adjust gel concentration
- Optimize sample preparation
- Ensure efficient transfer

TIP
Optimize each step of the workflow to achieve clear, sharp, and well-resolved bands.

08 Control Antibodies

Controls serve as internal references, typically using constitutively expressed housekeeping proteins to normalize protein loading and evaluate relative expression levels in Western blot analysis.

Catalog No.	Target	Species Reactivity	Application
E-AB-40337	GAPDH Polyclonal Antibody	Human, Mouse, Rat	WB, IHC
E-AB-48016	GAPDH Monoclonal Antibody	Human, Mouse, Rat , Bovine, Caprine, Guinea pig, Rabbit, Porcine	WB, IF
E-AB-48017	GAPDH Monoclonal Antibody	Zebrafish	WB
AN005280L	GAPDH Monoclonal Antibody	Human, Mouse, Rat	WB, IHC
AN004430L	Recombinant GAPDH Monoclonal Antibody	Human, Mouse, Rat	WB, IHC, IF
AN00296HP	HRP-conjugated GAPDH Monoclonal Antibody	Human, Mouse	WB
E-AB-40338	beta actin Polyclonal Antibody	Human, Mouse, Rat	WB, IHC
E-AB-48018	beta actin Monoclonal Antibody	Human, Mouse, Rat, Zebrafish	WB, IF
AN005290L	beta actin Monoclonal Antibody	Human, Mouse, Rat	WB, IHC, IF
AN004840L	Recombinant beta actin Monoclonal Antibody	Human, Mouse, Rat	WB, IHC, IF
AN00295HP	HRP-conjugated beta actin Monoclonal Antibody	Human, Mouse, Rat	WB
E-AB-20093	beta Tubulin Monoclonal Antibody	Human, Mouse, Rat	WB, IF
E-AB-40518	beta Tubulin Polyclonal Antibody	Human, Mouse, Rat	WB, IF
E-AB-20033	beta Tubulin Monoclonal Antibody	Human, Mouse, Rat, Monkey, Chicken, Dog, Hamster, Rabbit, Sheep, Insect, Yeast	WB, IHC-p, IF
E-AB-20043	beta Tubulin Monoclonal Antibody	Human, Mouse, Rat	WB
AN005310L	beta Tubulin Monoclonal Antibody	Human, Mouse, Rat, Zebrafish	WB, IF
AN00293HP	HRP-conjugated beta Tubulin Monoclonal Antibody	Human, Mouse, Rat, Zebrafish	WB
E-AB-48012	alpha Tubulin Monoclonal Antibody	Human, Mouse, E.coli, Scallop, Zebrafish	WB
E-AB-40510	alpha Tubulin Polyclonal Antibody	Mouse, Rat, Zebrafish	WB
E-AB-20036	alpha Tubulin Monoclonal Antibody	Human, Mouse, Rat	WB, IHC-p, IF, IP
AN005320L	alpha Tubulin Monoclonal Antibody	Human, Mouse, Rat	WB, IF
AN005340L	Lamin A/C Monoclonal Antibody	Human	WB, IF
AN00298HP	HRP-conjugated Lamin A/C Monoclonal Antibody	Human	WB
E-AB-40257	Lamin B1 Polyclonal Antibody	Human, Mouse, Rat	WB, IHC, IF
AN005330L	Lamin B1 Monoclonal Antibody	Human	WB, IF
E-AB-20076	Lamin B1 Monoclonal Antibody	Human, Mouse, Rat	WB, IHC-p, IF, IP
AN00292HP	HRP-conjugated Lamin B1 Monoclonal Antibody	Human	WB
AN005350L	PCNA Monoclonal Antibody	Human, Mouse, Rat	WB, IP, IHC
AN00472HP	HRP-conjugated PCNA Monoclonal Antibody	Human, Mouse, Rat	WB
AN005380L	VDAC1 Monoclonal Antibody	Human, Mouse, Rat	WB, IHC
AN005300L	Vinculin Monoclonal Antibody	Human, Mouse, Rat	WB, IHC, IF
AN00297HP	HRP-conjugated Vinculin Monoclonal Antibody	Human, Mouse, Rat	WB
AN005360L	YY1 Monoclonal Antibody	Human, Mouse, Rat	WB

09 Tag Antibodies

Tag antibodies specifically recognize epitope tags fused to target proteins, enabling reliable detection, quantification, and localization analysis in protein expression studies.

Catalog No.	Target	Species Reactivity	Application
E-AB-48008	Avi-Tag Monoclonal Antibody	All	WB
E-AB-48001	HRP-AVI-Tag Monoclonal Antibody	All	WB
E-AB-48025	Flag-Tag Monoclonal Antibody	All	WB, IF, IP, FCM
AN00299HP	HRP-conjugated Flag-Tag Monoclonal Antibody	All	WB
E-AB-48010	GFP Monoclonal Antibody	All	WB, IF, IP, FCM
E-AB-48043	GFP Monoclonal Antibody	All	WB, IF, IP
E-AB-48011	GFP-Tag Monoclonal Antibody	All	WB, IF
AN00432HP	HRP-conjugated GFP-Tag Monoclonal Antibody	All	WB
E-AB-48005	GST-Tag Monoclonal Antibody	All	WB
AN00431HP	HRP-conjugated GST-Tag Monoclonal Antibody	All	WB
AN004330L	Halo-Tag Monoclonal Antibody	All	WB
E-AB-48021	HA-Tag Monoclonal Antibody	All	WB, IF, IP, FCM
E-AB-40523	HA-Tag Polyclonal Antibody	All	WB, IF, IP, FCM
E-AB-48003	His-Tag Monoclonal Antibody	All	WB, IP, FCM
E-AB-40506	His-Tag Polyclonal Antibody	All	WB
E-AB-20013	MBP-Tag Monoclonal Antibody	All	WB
E-AB-48007	mCherry-Tag Monoclonal Antibody	All	WB, IF
E-AB-40508	mCherry-Tag Polyclonal Antibody	All	WB
E-AB-48024	Myc-Tag Monoclonal Antibody	All	WB, IF, IP
E-AB-48023	Myc-Tag Monoclonal Antibody	All	WB, FCM
E-AB-40524	Myc-Tag Polyclonal Antibody	All	WB
AN00300HP	HRP-conjugated Myc-Tag Monoclonal Antibody	All	WB
E-AB-20016	RFP-Tag Monoclonal Antibody	All	WB
E-AB-48020	S-tag Monoclonal Antibody	All	WB
E-AB-40522	S-tag Polyclonal Antibody	All	WB
E-AB-48009	Strep-Tag II Monoclonal Antibody	All	WB
E-AB-20021	Strep-Tag Monoclonal Antibody	All	WB
E-AB-40553	Sumo-Tag Polyclonal Antibody	All	WB
E-AB-20017	T7-Tag Monoclonal Antibody	All	WB
E-AB-40551	TRX Tag Polyclonal Antibody	All	WB
E-AB-48004	V5-Tag Monoclonal Antibody	All	WB
E-AB-40507	V5-Tag Polyclonal Antibody	All	WB
E-AB-20011	VSV-G-Tag Monoclonal Antibody	All	WB, IF, IP

10 Citations

Citations of Elabscience® Products in Western Blot Solutions

1. Li B, Wang W, Zhao L, et al. Photothermal therapy of tuberculosis using targeting pre-activated macrophage membrane-coated nanoparticles[J]. *Nature Nanotechnology*, 2024, 19(6): 834-845.
   Cited Product: RIPA Lysis Buffer (Strong) (Cat.:E-BC-R327)

2. Chen L, Guan W J, Qiu Z E, et al. SARS-CoV-2 nucleocapsid protein triggers hyperinflammation via protein-protein interaction-mediated intracellular Cl− accumulation in respiratory epithelium[J]. *Signal Transduction and Targeted Therapy*, 2022, 7(1): 255.
   Cited Product: RIPA Lysis Buffer (Strong) (Cat.:E-BC-R327)

3. Zeng H, Pan T, Zhan M, et al. Suppression of PFKFB3-driven glycolysis restrains endothelial-to-mesenchymal transition and fibrotic response[J]. *Signal Transduction and Targeted Therapy*, 2022, 7(1): 303.
   Cited Product: Skim Milk Powder (Cat.:E-BC-R337)

4. Huang Q, Sun Y, Peng L, et al. Extracellular vesicle‐packaged ILK from mesothelial cells promotes fibroblast activation in peritoneal fibrosis[J]. *Journal of Extracellular Vesicles*, 2023, 12(7): 12334.
   Cited Product: ECL Substrate Detection Kit (Cat.:E-BC-R347)

5. Hou P, Zhu H, Chu F, et al. Phenazine biosynthesis-like domain-containing protein (PBLD) and Cedrelone promote antiviral immune response by activating NF-κB[J]. *Nature Communications*, 2025, 16(1): 496.
   Cited Product: 5× SDS Loading Buffer(Cat.:E-BC-R288)

6. Li Y, Wang J, Yu R, et al. Leveraging chemotherapy-induced PD-L1 upregulation to potentiate targeted PD-L1 degradation using nanoparticle-based targeting chimeras[J]. *Chemical Engineering Journal*, 2024, 499: 155708.
   Cited Product: Excellent Chemiluminescent Substrate (ECL)Diagnostic Kit(Cat.:E-IR-R307)

7. Zhang Y, Xia Q, Wu T, et al. A novel multi-functionalized multicellular nanodelivery system for non-small cell lung cancer photochemotherapy[J]. *Journal of Nanobiotechnology*, 2021, 19(1): 245.
   Cited Product: Western Blot Detection Kit(Cat.:E-IR-R304A)

8. Wang L, Liang L, Liu B, et al. A controlled T7 transcription-driven symmetric amplification cascade machinery for single-molecule detection of multiple repair glycosylases[J]. *Chemical Science*, 2021, 12(15): 5544-5554.
   Cited Products: Western Blot Detection Kit(Cat.:E-IR-R304A), ECL Substrate Detection Kit (Cat.:E-BC-R347)

9. Shen Z, Liu Z, Sun L, et al. Constructing epidermal rete ridges using a composite hydrogel to enhance multiple signaling pathways for the maintenance of epidermal stem cell niche[J]. *Acta Biomaterialia*, 2023, 169: 273-288.
   Cited Products: SDS-PAGE Gel kit (Cat.:E-IR-R305), Excellent Chemiluminescent Substrate (ECL)Diagnostic Kit(Cat.:E-IR-R307)

10. Wang L, Lu Y, Zhang C. Construction of a self-directed replication system for label-free and real-time sensing of repair glycosylases with zero background[J]. *Chemical Science*, 2020, 11(2): 587-595.
    Cited Products: Western Blot Detection Kit(Cat.:E-IR-R304A), ECL Substrate Detection Kit (Cat.:E-BC-R347)



Elabscience Bionovation Inc.

Toll-free: 1-888-852-8623
Tel: 1-832-243-6086
Fax: 1-832-243-6017
Web: www.elabscience.com
Email: orders@elabscience.com; techsupport@elabscience.com

EBR139-2609v1