HISTORY OF Western Blotting

2013
Western blot signal enhancer reagent (MilliporeSigma) was used to successfully detect proteins from the blood of just 6 zebrafish.

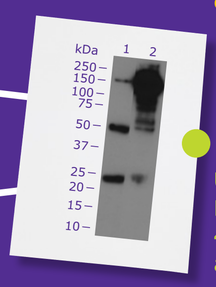
2008
SNAP i.d.® vacuum-driven system (MilliporeSigma) reduced immunodetection time (blocking, probing, washing) to 30 minutes

2005
Launch of Immobilon®-FL Low Autofluorescence PVDF Membrane, now the most popular transfer membrane for fluorescent Western blots

1999
Birth of quantitative Western blots using fluorescent probes conjugated to antibodies

1992
Far western blot first used to detect protein-protein interactions

Kaelin, W.G. Jr. et al. Expression cloning of a cDNA encoding a retinoblastoma-binding protein with E2F-like properties. Cell 70, 351–364



1989
Upstate, now part of MilliporeSigma, developed 4G10® anti-phosphotyrosine antibody, launching cell signaling research

Circa 1986
First commercially available precast gels for SDS-PAGE

1985
Immobilon® PVDF Membrane invented by MilliporeSigma, specifically for use in Western blotting

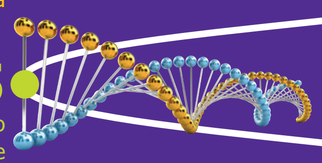
Pluskal, Malcolm G.; Przekop, Michelle B.; Kavonian, Mark R.; Vecoli, Charles; Hicks, David A. BioTechniques (1986), 4(3), 272-83.

1979
Western blotting technique published by Harry Towbin, Theophil Staehelin and Julian Gordon using nitrocellulose membrane from MilliporeSigma

1975
Monoclonal antibodies developed to target specific antigens

Köhler, G., and Milstein, C. (1975). Continuous cultures of fused cells secreting antibody of predefined specificity. Nature 256, 495–497.

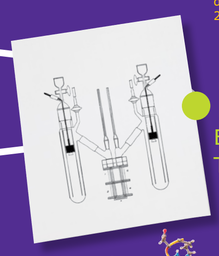
1975
Southern blot invented to detect DNA, using nitrocellulose membrane from MilliporeSigma



1970
Sodium dodecyl sulfate polyacrylamide gel electrophoresis established

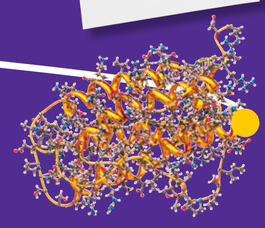
Laemmli, U.K., 1970. Cleavage of structural proteins during assembly of the head of bacteriophage T4. Nature 227, 680.

1941
Albert Coons discovered that fluorophore-labeled antibodies retained specific binding properties



1937
Birth of electrophoresis: Tiselius apparatus

1890
Antibody activity discovered: Kitasato Shibasaburô



1747
First protein discovered—wheat gluten

MilliporeSigma

TIMELESS
Recipes and Solutions for Blotting Success

Standard recipes for Western blotting

TBS 10x (concentrated Tris-buffered saline)
200 mM Tris
1500 mM NaCl

For 1 L:
24 g Tris base (formula weight: 121.1 g)
88 g NaCl (formula weight: 58.4 g)
Dissolve in 900 mL Milli-Q® water
pH to 7.6 with 12 N HCl
Add Milli-Q® water to a final volume of 1 L

1X solution: mix 1 part of the 10X solution with 9 parts Milli-Q® water and adjust pH to 7.6.

TBST (Tris-buffered saline, 0.1% Tween® 20)
For 1 L:
100 mL of TBS 10X
900 mL of Milli-Q® water
1 mL Tween® 20

4X SDS-PAGE sample loading buffer
1.5 mL of 1 M Tris-HCl pH 6.8
3 mL of 1 M DTT (dithiothreitol)
0.6 g of SDS (sodium dodecyl sulfate)
0.03 g of bromophenol blue
2.4 mL of glycerol
Bring final volume to 7.5 mL with Milli-Q® water
If solution is orange/yellow in color, add 1 drop of 5 M NaOH to adjust pH
Make 500 µL aliquots and store at -20°C

SDS-PAGE gel making buffer
1.5 M Tris-HCl (for separating gel)
118.2 g of Tris-HCl,
Add 450 mL Milli-Q® water and adjust pH to 8.8
Add Milli-Q® water to final volume 500 mL
Filter and degas

SDS-PAGE gel making buffer
1 M Tris-HCl (for stacking gel)
78.8 g of Tris-HCl
Add 450 mL Milli-Q® water and adjust pH to 6.8
Add Milli-Q® water to final volume 500 mL
Filter and degas

SDS-PAGE 10X gel running buffer
60 g Tris base (248 mM)
288 g glycine (1.92 M)
20 g SDS (1% w/v)
Milli-Q® water to final volume 2 L
No need to pH, filter, or degas
Dilute to 1X for running SDS-PAGE gels

10X PBS (1 L)
81.8 g NaCl (1.4 M)
20.1 g KCl (270 mM)
14.2 g Na_2HPO_4 (100 mM)
2.45 g KH_2PO_4 (18 mM)
pH 7.4
Add Milli-Q® water to final volume of 1 L

Transfer buffer 10X
15.2 g Tris base
72.1 g glycine
5.0 g SDS
Milli-Q® water to final volume 500 mL

Transfer buffer 1X (make fresh each time)
50 mL 10X transfer buffer
100 mL methanol
Milli-Q® water to final volume 500 mL

SDS-PAGE Coomassie® staining solution
1.25 g Coomassie® blue R-250
225 mL methanol
225 mL Milli-Q® water
50 mL glacial acetic acid

SDS-PAGE destaining solution
300 mL methanol (30%)
100 mL acetic acid (10%)
600 mL Milli-Q® water

Recipes for TruPAGE™ Precast Gel System

20X TruPAGE™ TEA-Tricine SDS Running Buffer
179.0 g triethanolamine
143.3 g tricine
20 g SDS
Milli-Q® water to final volume 1.0 L
The pH should be between 8.2–8.3 at 25 °C.

20X TruPAGE™ Tris-MOPS SDS Express Running Buffer
145.2 g Tris base
143.3 g MOPS
20 g SDS
Milli-Q® water to final volume 1.0 L
The pH should be between 8.2–8.3 at 25 °C.
NOTE: Antioxidant (5 mM sodium bisulfite) is required in the inner chamber if using this buffer.

4X TruPAGE™ LDS Sample Buffer
4.0 g glycerol
0.40 g lithium dodecyl sulfate
1.2 g triethanolamine
0.40 g Ficoll® 400
2.5 mg Phenol Red
2.5 mg Brilliant Blue G250
7.0 mg EDTA
Milli-Q® water to final volume 10 mL
The pH should be between 7.7-7.8 at 25 °C.
Dissolve triethanolamine (0.8 M) and pH to 7.6 before adding other reagents.

800X TruPAGE™ Running Antioxidant
4.2 g Sodium Bisulfite
Milli-Q® water to final volume 10 mL

20X TruPAGE™ Transfer Buffer
30.3 g Tris base
144.1 g glycine
Milli-Q® water to final volume 1.0 L.

10X Dithiothreitol sample reducer
38.6 mg DTT
Milli-Q® water to final volume 1.0 mL

Prepare and Grow

- Cells, media, sera, cultureware
- Stericup® media filtration devices

Precise Westerns start with low-binding, high recovery Stericup® vacuum filters – for media volumes of 150 mL –1,000 mL

Collect and Extract

- ProteoExtract® kits for fractionation
- CytoBuster™, BugBuster™ and CelLytic™ protein extraction reagents
- Benzonase® nuclease
- cOmplete™ and SIGMAFAST™ protease inhibitors

Maintain and preserve protein functionality following cell lysis using individual protease inhibitors and protease inhibitor cocktails.

Enrich and Concentrate

- Amicon® filters for concentration and buffer exchange
- PureProteome® magnetic beads for enrichment and purification
- Recombinant protein purification affinity gels

Unlike methods that require careful aspiration to avoid sample loss, PureProteome® magnetic beads are isolated using a magnetic rack, for total removal of buffers and complete recovery with no sample dilution.

Qualify and Electrophorese

- TruPAGE™ precast gels
- Buffers (including Trizma® Tris base), solvents, prestained MW markers
- Direct Detect® IR spectrometer for protein sample qualification

TruPAGE® precast gels are tear-resistant to reduce handling failures that commonly occur during gel electrophoresis and downstream processing.

Blot and Analyze

- Range of Immobilon® PVDF transfer membranes
- Western-validated antibodies
- SNAP i.d.® 2.0 protein detection system
- Immunodetection reagents

Blot efficiently using our Western blotting reagents, including Bløk® noise-cancelling reagents, Luminata™ Western HRP substrates, Immunoreaction Enhancer Reagents, and ReBlot™ Plus antibody stripping kits, all carefully optimized for your success.

www.sigma.com/westernblot

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