

Sample Preparation

Lysis buffer & sonication = better release of proteins

[Cell Lysis Buffers](#)

[PBS](#)

[Other Buffers](#)

[Western Blotting Application Solutions Kit](#)

[Cell Fractionation Kits](#)

[Activators & Inhibitors](#)

⋮



SDS-PAGE

Run with appropriate controls

[Buffers](#)

[Control Cell Extracts & Proteins](#)

[Protein Molecular Weight Markers](#)

[Loading Controls](#)



Transfer

To nitrocellulose membranes

[Nitrocellulose Sandwiches](#)

[Wet Transfer Buffer](#)

[Ponceau S Staining Solution](#)



Blocking

5% milk in TBST

[Milk & Blocking Solutions](#)

[Tris Buffered Saline with Tween® 20 \(TBST-10X\)](#)



Primary Antibody Incubation

At 4°C overnight in 5% BSA or nonfat milk* in TBST

[Tris Buffered Saline with Tween® 20 \(TBST-10X\)](#)

[Primary Antibodies](#)

[Primary Antibody Incubation Buffers:](#)

[Milk](#)

[Animal-Free Blocking Solution](#)

[BSA](#)

⋮



Detection

Mix reagents immediately before use for chemiluminescent detection

[Detection Reagents](#)



Washing

3 x 5 min in TBST

[Tris Buffered Saline with Tween® 20 \(TBST-10X\)](#)



Secondary Antibody Incubation

For 1 hr at room temperature in 5% nonfat milk in TBST

[Secondary Antibodies](#)

[BSA](#)

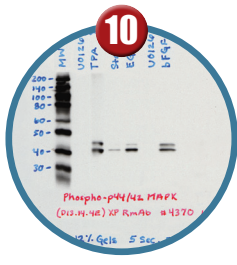
[Tris Buffered Saline with Tween® 20 \(TBST-10X\)](#)



Washing

3 x 5 min in TBST

[Tris Buffered Saline with Tween® 20 \(TBST-10X\)](#)



Imaging

Visualization of western blot results

10 Steps to a Better Western Blot

Although western blotting is widely used and accepted, problems with experiment set up and selection of reagents can lead to suboptimal results, which can be time consuming and frustrating. This guide will help you improve your western blotting with the protocol and products to get the best results in the shortest amount of time. You'll find recommendations for reagents and procedures based on our extensive experience with western blotting, which is part of our validation, lot testing, and technical support processes.

*as recommended on product datasheet



cst-science.com/WBprotocol

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