10X Western Blot Lysis Buffer

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Catalog #: 100-LYS **Size:** 15 mL

Product Description: 10X Western Blot Lysis Buffer optimized to fully solubilize proteins in preparation for western blotting. May be used for organs and tissue culture cells.

Background: Nonionic detergents such as NP-40 or triton fail to solubilize many cellular proteins involved in cell signaling. This problem is particularly acute in brain where synaptic junctions are known to be insoluble in nonionic detergents. 1% SDS completely solubilizes membrane and other hard to solubilize proteins including synaptic junction proteins.

*SDS inactivates most proteases, but additional protease inhibitors can be added as needed before use as some proteases are insensitive to or even activated (e.g. proteinase K) by SDS.

*1% SDS inactivates phosphatase enzymes. If treating your lysate with phosphatase enzymes this lysis buffer is not recommended.

Packaging: 15 mL of 10X Western Blot Lysis Buffer consisting of 10% (w/v) SDS, 100mM TRIS, 10mM EDTA, pH 8.0. Dilute to 1X before use.

Storage and Stability: Shipped at room temperature. Storage at room temperature is recommended for both 10X and 1X solutions to prevent SDS from precipitating out of solution.

Lysate Preparation Protocol using 1X Western Blot Lysis Buffer:

Prepare Samples

A. Organs- Whole cell lysate

1. Place organ in conical tube or microfuge tube with screw cap. For large organs cut into 1/4"sections. For organs that are thick and dense cut even smaller sections.
2. Add lysis buffer to organs in conical tube completely submerging the organ in the lysis buffer. Keep sample at room temperature.

B. Adherent Tissue Culture Cells

1. Wash cells with 2-10 mL of room temperature 1X PBS once. Remove wash with transfer pipet.
2. Add lysis buffer, enough to cover the entire plate.
3. Rock and rotate the plate to thoroughly coat cells.
4. Transfer to a conical tube or microfuge tube with screwcap.

C. Suspension TC Cells

1. Pipette media and cells into conical tube.
2. Pellet cells by centrifugation (1 minute at 1800 x *g)* and remove media.
3. Resuspend cells with 1-3 mL of room temperature 1X PBS to wash.
4. Repeat step #2.
5. Add lysis buffer.

Lyse Samples

1. Sonicate sample in 5-20 second intervals until buffer is clear and can be easily pipetted without clogging.
2. Heat sample at 95C for 10 minutes. Cool to room temperature.
3. Centrifuge lysate at 1800 x *g* for 5 minutes to pellet cell debris.
4. Pipette lysate into fresh conical or microfuge tube.

For additional tech tips regarding this product visit our website!

https://www.phosphosolutions.com/protocols-lysate-preparation/