WB Lysis Buffer

Cat #: BMP2010 Size: 100 mL

	WB Lysis Buffer
REF	**Cat #:** BMP2010 | LOT | **Lot #:** Refer to product label
	Applicable samples: Animal Tissues, Cells
	Application: SDS-PAGE, WB
	Storage: Store according to the recommended storage conditions of each component, stable for 12 months

Assay Principle

WB Lysis Buffer is a lysate under denaturing conditions. It is mainly used to extract soluble proteins from animal cells and tissues, which has strong lysis effects on cell membrane, cytoplasm and nucleus components. The protein sample lysed can be used for conventional SDS-PAGE, WB and other experiments. Stored at 4℃ without thawing. It is a fast and convenient cell tissue lysis solution.

Materials Supplied and Storage Conditions

Kit components	Size	Storage conditions
WB Lysis Buffer	50 mL×2	4℃
PMSF (100 mM)	1.5 mL	-20℃, protected from light

Materials Required but Not Supplied

- Ice Maker, Refrigerated Centrifuge, Vortex
- Protease Inhibitor Cocktail (100×)
- Phosphatase Inhibitor Cocktail
- Precision Pipettes, Disposable Pipette Tips
- Dounce homogenizer (for Tissue Samples)

Reagent Preparation

WB Lysis Buffer: Ready to use as supplied. Keep on ice before use. Store at 4℃.
PMSF (100 mM): Ready to use as supplied. Keep on ice before use. Store at -20℃, protected from light.
Working WB Lysis Buffer: Before use, take an appropriate amount of WB Lysis Buffer (approximately 50-100 μL per 1 $\times 10^6$ cells or 150-250 μL per 20 mg tissue sample) and add PMSF (100 mM) at 100:1 (V/V). Add Protease Inhibitor Coc ktail (100×) and Phosphatase Inhibitor Cocktail as needed.

Assay Procedure

A. For adherent cells

Abbkine, Inc • USA • ***www.abbkine.com***
Not for further distribution without written consent.Copyright © Abbkine, Inc.

1. Discard the medium and wash once with PBS, physiological saline or serum-free culture medium (if the protein in the serum does not interfere, it is not necessary to wash), and centrifuge again to collect the cells.
2. Add Working WB Lysis Buffer at a ratio of about 50-100 μL per 1×10 ^{6} cells. For example, about 150-250 μL Working WB Lysis Buffer should be added to each well of a 6-well plate. Use a pipette several times to make sufficient contact between Working WB Lysis Buffer and the cells. Usually, the cells are lysed after Working WB Lysis Buffer is in contact with the cells for 1-2 s.
3. After fully lysed, centrifuge at 10,000-14,000 g for 3-5 min, and take the supernatant for subsequent SDS-PAGE and WB.

B. For tissue samples

1. Cut the tissue into fine pieces.
2. Add Working WB Lysis Buffer at a ratio of 150-250 μL per 20 mg tissue sample.
3. Homogenize with a homogenizer and lyse on ice for 3-5 min.

Note: 1. If the sample is not fully lysed, the amount of Working WB Lysis Buffer can be appropriately increased; if high-concentration protein samples are required, the amount of Working WB Lysis Buffer can also be appropriately decreased. 2. If the tissue sample is very small, it can be cut properly and directly added to Working WB Lysis Buffer, and fully lysed by vigorous vortexing.

4. After fully lysed, centrifuge at 10,000-14,000 g for 3-5 min, and take the supernatant for subsequent SDS-PAGE and WB operations.

Precautions

1. All steps of lysing samples should be performed on ice or at 4℃. The lysed protein samples can be aliquoted and stored at -80℃ for a long time.
2. When stored at 4℃, the SDS in WB Lysis Buffer is easy to separate out. Please put it at 37℃ to dissolve it completely before use, and it can be used after returning to room temperature.
3. The protein concentration of the cleaved protein sample can be determined with Protein Quantification Kit (BCA Assay). Due to the high concentration of interfering substances such as Triton X-100, the protein concentration of the sample cannot be determined by the Bradford method.
4. A small group of transparent jelly often appears in the lysate of WB Lysis Buffer, which is a normal phenomenon. This transparent gel is a complex containing genomic DNA and the like. In the case of not detecting proteins that are particularly tightly bound to genomic DNA, the supernatant can be directly centrifuged to take the supernatant for subsequent experiments. If you need to detect proteins that are particularly tightly bound to the genome, you can break up the transparent gelatinous substance by sonication, and then centrifuge the supernatant was taken for subsequent experiments. If some common transcription factors are detected, such as NF-kappaB, p53, etc., the detection can usually be completed without sonication.

Recommended Products

Catalog No.	Product Name
KTP3007	ExKine™ Pro Total Protein Extraction Kit for Animal Cultured Cells/Tissues
KTD3001	Protein Quantification Kit (BCA Assay)
BMP1001	Protease Inhibitor Cocktail (100×)
KTD3003	SDS-PAGE Protein Sample Loading Buffer (5×)

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.

Not for further distribution without written consent.Copyright © Abbkine, Inc.