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ab179835 Mammalian Cell Lysis Buffer 5X

For simple and rapid preparation of mammalian cell lysates.

View kit datasheet: www.abcam.com/ab179835
(use www.abcam.cn/ab179835 for China, or www.abcam.co.jp/ab179835 for Japan)

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Mammalian Cell Lysis Buffer 5X (ab179835) is widely used to prepare mammalian cells lysates for use in a variety of downstream biochemical assays, especially those for quantification of enzymatic activity. This buffer does not contain SDS, a reagent that is now known to interfere with many activity tests.

The buffer just requires a simple 5-fold dilution, and minimal hands-on-time. Cell lysates are ready in only 15 minutes.

2. Protocol Summary

Treat sample as desired; wash in PBD

Lyse sample in 1X Lysis Buffer

Sample is ready for use

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at 4°C in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition (Before prep)	Storage Condition (After prep)
Mammalian Cell Lysis Buffer	10 mL	4°C	4°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- MilliQ water or other type of double distilled water (ddH_2O)
- PBS
- Pipettes and pipette tips, including multi-channel pipette
- Assorted glassware for the preparation of reagents and buffer solutions
- Tubes for the preparation of reagents and buffer solutions
- General cell culture consumables and instrumentation
- Dounce homogenizer (if using tissue)
- (Optional) Protease inhibitors: we recommend Protease Inhibitor Cocktail II (ab201116) [AEBSF, aprotinin, E-64, EDTA, leupeptin] as general use cocktail.

8. Technical Hints

- **This kit is sold based on number of tests. A "test" simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample and reagent additions.
- Ensure plates or tubes are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 Mammalian Cell Lysis Buffer 5X:

Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

Prior to use, prepare a 1X Cell Lysis Buffer by diluting 1 mL of Mammalian Cell Lysis Buffer 5X into 4 mL of ddH_2O. Mix well.

Δ Note: 5 mL of 1X Cell Lysis Buffer provides enough reagent to lyse 1 x 96 well plate.

10. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- If the downstream assay you will perform requires the addition of protease inhibitors, add them to the 1X Lysis Buffer immediately prior use.

10.1 Adherent cell samples:

10.1.1 Grow and treat cells as required in your desired culture vessel to about ~80% confluence.
10.1.2 Wash cells in PBS to remove residual media.
10.1.3 Lysis cells with 1X Lysis Buffer. Table below indicates suggested volumes.

Plate	384-wp	96-wp	48-wp	24-wp	12-wp	6-wp	100 cm^2
Volume	20 µL	50 µL	100 µL	150 µL	200 µL	300 µL	800 µL

10.1.4 Incubate cells at room temperature for 10 – 20 minutes.
10.1.5 Centrifuge lysate at 1500 rpm for 5 minutes.
10.1.6 Transfer supernatant to a new tube.
10.1.7 Keep on ice.

10.2 Suspension cell samples:

10.2.1 Grow and treat cells as required in your desired culture vessel.
10.2.2 Wash cells in PBS to remove residual media.
10.2.3 Add 100 µL of 1X Lysis Buffer to 1 – 5 x 10^6 cells.
10.2.4 Incubate cells at room temperature for 10 – 20 minutes.
10.2.5 Centrifuge lysate at 1500 rpm for 5 minutes.
10.2.6 Transfer supernatant to a new tube.
10.2.7 Keep on ice.

10.3 Tissue samples:

10.3.1 Harvest 20 mg tissue.
10.3.2 Wash tissue with cold PBS.
10.3.3 Homogenize tissue with 400 µL of 1X Lysis Buffer.
Homogenization can be done using a Dounce homogenizer or pestle, sitting on ice, with 10 – 15 passes.
10.3.4 Centrifuge at 2500 rpm for 5 – 10 minutes.
10.3.5 Transfer supernatant to a new tube.
10.3.6 Keep on ice.

10.4 Plant cell samples:

10.4.1 Homogenize leave with 1X Lysis Buffer at final concentration of 200 mg/mL.
Homogenization can be done using a Dounce homogenizer or pestle, with 10 – 15 passes.
10.4.2 Centrifuge at 2500 rpm for 5 – 10 minutes.
10.4.3 Transfer supernatant to a new tube.
10.4.4 Keep on ice.

10.5 Bacterial cell samples:

10.5.1 Collect bacterial cells by centrifugation at 10,000 x g at 4°C for 15 minutes.
10.5.2 Use 10^7 – 10^8 cells/mL of 1X Lysis Buffer.
10.5.3 Incubate solution at room temperature for 15 minutes.
10.5.4 Centrifuge at 2500 rpm for 5 minutes.
10.5.5 Transfer supernatant to a new tube.

11. Notes

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