

**(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !)**

## RIPA Lysis Buffer (Strong)

**Catalog No:** E-BC-R327

**Sizes:** 20 mL/ 50 mL/ 100 mL

Cat	Product	20 mL	50 mL	100 mL	Storage
E-BC-R327	RIPA Lysis(Strong)	20 mL	50 mL	100 mL	-20 ℃
E-BC-R287	100 mM PMSF	200 µL	500 µL	1 mL	-20 ℃
E-BC-R250	100 mM Na <sub>3</sub> VO <sub>4</sub>	200 µL	500 µL	1 mL	-20 ℃
<b>Manual</b>			<b>1 copy</b>		

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help.

Phone: 240-252-7368(USA) 240-252-7376(USA)

Email: [techsupport@elabscience.com](mailto:techsupport@elabscience.com)

Website: [www.elabscience.com](http://www.elabscience.com)

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

## Introduction

RIPA Lysis Buffer is a traditional rapid cell tissue lysate used as the preferred lysate for protein extraction from tissues or cells in the Western Blot assay.

## Instructions

### 1. For Tissue Samples

- a. Take the samples, wash the tissue thoroughly with pre-cooled PBS (0.01M, pH7.4) to remove the surface blood and internal debris.
- b. Weigh and smash the tissue, add an appropriate ratio of RIPA Lysis Buffer (add 10  $\mu$ L PMSF and 10  $\mu$ L  $\text{Na}_3\text{VO}_4$  to 1 mL RIPA Lysis) and homogenize the tissue.  
It is recommended to homogenize according to the ratio of tissue weight: RIPA volume = 3:10. For example, add 1 mL RIPA Lysis Buffer to 0.3 g tissue sample, the specific volume can be adjusted according to experimental requirements.
- c. Shake and lyse on the ice for 30 min after homogenization. And then sonicate the sample for 1 min (under ice water bath conditions) with 2 s' sonication and 2 s' intervals to make cells fully lysis and reduce the viscosity of sample.
- d. Centrifuge at 12,000 rpm for 10 min at 4  $^{\circ}\text{C}$ .
- e. Take the supernatant and measure the protein concentration.

### 2. For Cell Sample

- a. Collect the cells, wash them thoroughly with pre-cooled PBS (0.01 M, pH7.4) to remove the medium off (it is generally recommended to wash 3 times).
- b. Add an appropriate ratio of RIPA Lysis Buffer (10  $\mu$ L PMSF and 10  $\mu$ L  $\text{Na}_3\text{VO}_4$  in 1 mL RIPA Lysis) and lyse on the ice for 30 min.  
It is recommended to add 0.1 mL of RIPA Lysis Buffer to each well of a 6-well plates (the protein content in different cells may vary, and the volume of the lysate added can be appropriately adjusted).
- c. Sonicate the sample for 1 min (under ice water bath conditions) with 2 s' sonication and 2 s' intervals to make cells fully lyse and reduce viscosity of sample.
- d. Centrifuge at 12,000 rpm for 10 min at 4  $^{\circ}\text{C}$ .
- e. Take the supernatant and measure the protein concentration.

## RIPA Lysis Buffer components

50 mM Tris (pH7.4), 150 mM NaCl, 1% Triton X-100, 1%  $\text{C}_{24}\text{H}_{39}\text{O}_4\text{Na}$ , 1 mM EDTA, 0.1% SDS, 10 mM NaF, 1 mM  $\text{Na}_3\text{VO}_4$ , 1 mM PMSF.

## Storage

Store at -20 °C for 12 months.

## Cautions

1. For the protein extraction with RAPI Lysis Buffer, the whole process must be on the ice or at 4 °C.
2. It is recommended to add 10 µL PMSF and 10 µL Na<sub>3</sub>VO<sub>4</sub> to RAPI Lysis before use. If RIPA Lysis is crystalline state, dissolve at room temperature or in a warm water bath.
3. A cloud of transparent gels may appear in the lysis product, which is a normal phenomenon because the lysis product is a compound containing genomic DNA. It is recommended to sonicate the sample for seconds. Take the supernatant after centrifugation for subsequent testing.
4. The protein sample lysed by RIPA Lysis Buffer cannot be measured by the Bradford method because of the high concentration of detergent in the lysis. It is recommended to measure the protein concentration by the BCA method.
5. For your safety and health, please wear the lab coat and disposable gloves before the experiments.