

# Extraction Enhancer Buffer 50X ab193971

SimpleStep ELISA

## 4 References

### Overview

<b>Product name</b>	Extraction Enhancer Buffer 50X
<b>Product overview</b>	<p>Abcam's Extraction Enhancer Buffer 50X is formulated for the preparation of cell or tissues extracts for use in immunoassays. We recommend using ab193971 when having difficulty in pipetting lysate or viscous lysate.</p> <p>The Extraction Enhancer Buffer 50X should be diluted to 1X in the appropriate buffer before use. If required the Extraction Enhancer Buffer 50X can be used in conjunction of Abcam's Extraction Buffer 5X (<a href="#">ab193970</a>).</p> <p>The Extraction Enhancer Buffer 50X may precipitate when stored at + 4°C. To dissolve, warm briefly at + 37°C and mix gently.</p>

<b>Notes</b>	<p><b><u>Instructions for use</u></b></p> <p>If the Extraction Buffer 5X is to be used in conjunction with Extraction Enhancer Buffer 50X (ab193971), then follow the instructions below:</p> <p><i>Note: The provided Extraction Buffer 5X contains phosphatase inhibitors and protease inhibitor aprotinin. Additional protease inhibitors can be added if required.</i></p>
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### **1X Extraction Buffer + Enhancer**

Prepare 1X Extraction Buffer + Enhancer by diluting Extraction Buffer 5X and Extraction Enhancer Buffer 50X to 1X with deionized water. To make 10 mL 1X Extraction Buffer + Enhancer combine 7.8 mL deionized water, 2 mL Extraction Buffer 5X and 200 µL Extraction Enhancer Buffer 50X. Mix thoroughly and gently.

### **Sample preparation**

#### **Preparation of extracts from cell pellets**

Collect non-adherent cells by centrifugation or scrape to collect adherent cells from the culture flask. Typical centrifugation conditions for cells are 500 x g for 5 minutes at 4°C.

Rinse cells twice with PBS.

Solubilize pellet at  $2 \times 10^7$  cell/mL in chilled 1X Extraction Buffer or 1X Extraction Buffer + Enhancer.

Incubate on ice for 20 minutes. Centrifuge at 18,000 x g for 20 minutes at 4°C. Transfer the

supernatants into clean tubes and discard the pellets.

Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.

Dilute samples to desired concentration in 1X Extraction Buffer or 1X Extraction Buffer + Enhancer.

#### **Preparation of extracts from adherent cells by direct lysis (alternative protocol)**

Remove growth media and rinse adherent cells 2 times in PBS.

Solubilize the cells by addition of chilled 1X Extraction Buffer or 1X Extraction Buffer + Enhancer directly to the plate (use 750 µL - 1.5 mL 1X Extraction Buffer or 1X Extraction Buffer + Enhancer per confluent 15 cm diameter plate).

Scrape the cells into a microfuge tube and incubate the lysate on ice for 15 minutes. Centrifuge at 18,000 x g for 20 minutes at 4°C.

Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.

Dilute samples to desired concentration in 1X Extraction Buffer or 1X Extraction Buffer + Enhancer.

#### **Preparation of extracts from tissue homogenates**

Tissue lysates are typically prepared by homogenization of tissue that is first minced and thoroughly rinsed in PBS to remove blood (dounce homogenizer recommended).

Homogenize 100 to 200 mg of wet tissue in 500 µL – 1 mL of chilled 1X Extraction Buffer or 1X Extraction Buffer + Enhancer. For lower amounts of tissue adjust volumes accordingly. Incubate on ice for 20 minutes.

Centrifuge at 18,000 x g for 20 minutes at 4°C.

Transfer the supernatants into clean tubes and discard the pellets.

Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.

Dilute samples to desired concentration in 1X Extraction Buffer or 1X Extraction Buffer + Enhancer.

### **Properties**

#### **Storage instructions**

Store at +4°C. Please refer to protocols.

Components	1 ml	5 ml
50X Cell Extraction Enhancer Solution (ab193971)	1 x 1ml	1 x 5ml

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