ab65398

Cytosol/Particulate Rapid Separation Kit

Instructions for Use

For the easy and rapid separation of cytosol from particulates in cell and tissue culture.

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

The location and translocation of proteins, signaling molecules, and other small molecules inside cells regulate cell growth, differentiation and many other cellular functions. Separating cytosol and particulate fractions is an important step in studying subcellular localization of cellular components. However, traditional methods usually take hours to separate cytosol from particulates, so that some cellular components especially small molecules and metabolites are getting diffused or redistributed during the separation procedure.

Abcam’s Cytosol/Particulate Rapid Separation Kit physically separate cytosol from particulate compartments rapidly through an oil layer and thus the two fractions would not contact or diffuse to each other. Using the method, contaminations can be avoided even for small molecules. Subcellular localization and analyses of factors interested can be performed accurately.
2. Protocol Summary

- Prepare Oil-Particulate Layers
- Suspend Cells in Cytosol Releasing Buffer
- Apply Cell Suspension to Oil-Particulate Layers
- Incubate on Ice
- Collect Cytosol Fraction and Particulate Fraction
- Store at -80°C for Further Use
3. Components and Storage

A. Kit Components

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell Suspension Buffer</td>
<td>2 mL</td>
</tr>
<tr>
<td>Cytosol Releasing Buffer</td>
<td>2 mL</td>
</tr>
<tr>
<td>Oil Layer</td>
<td>25 mL</td>
</tr>
<tr>
<td>Particulate Layer</td>
<td>2 mL</td>
</tr>
</tbody>
</table>

* Store kit at -20°C.

- Store Cell Suspension Buffer, Oil Layer and Particulate Layer at +4°C.
- Store Cytosol Releasing Buffer at -20°C.
- Keep all kit components on ice at all times during the experiment.
B. Additional Materials Required

- Microcentrifuge
- Pipettes and pipette tips
- Orbital shaker
4. Assay Protocol

The following protocol is described for fractionation of about $2 \times 10^6$ cells. If more cells are needed for fractionation, scale up the volume proportionally.

If desired, protease inhibitors can be added to the Cytosol Releasing Buffer to prevent protein degradations.

1. Prepare Oil-Particulate Layers in a microcentrifuge tube:
   Add 40 $\mu$l Particulate Layer into a microcentrifuge tube, and then add 0.5 ml Oil Layer on top of the Particulate Layer. Do not mix. Keep on ice.

2. Collect cells by centrifugation at 600 x $g$ for 5 minutes at 4°C.

3. Re-suspend cells (~2 x $10^6$ cells) in 40 $\mu$l Cell Suspension Buffer.

4. Add 40 $\mu$l Cytosol Releasing Buffer. Pipette up and down to mix well.

5. Apply the sample on top of the Oil-Particulate Layers prepared in Step 1 (do not mix samples with the Oil-Particulate Layers). Incubate on ice for a total 30 seconds from the time point of adding Cytosol Releasing Buffer to the cell suspensions (i.e. from Step 4).
**Note:**

The time that cells interact with Cytosol Releasing Buffer is critical. 30 seconds appear to be optimal. Shorter incubation time may result in incomplete release of cytosol, whereas longer incubation time may result in contaminations.

6. Spin the tube in a microcentrifuge at top speed for 1 minute. The cytosol and particulate fractions should be physically separated by the middle Oil Layer.

7. Collect the Cytosol Fraction (top layer) into a fresh tube. Collect the Particulate Fraction (bottom layer: Particulate layer and pellet) into a separate tube.

8. Store both fractions at -80°C for further analyses. Generally, 30-40% proteins are in the cytosol fraction.

**Note:**

If Oil Layer was taken into the fractions, the fraction may be centrifuged again to remove oil.

For further technical questions please do not hesitate to contact us by email ([technical@abcam.com](mailto:technical@abcam.com)) or phone (select “contact us” on [www.abcam.com](http://www.abcam.com) for the phone number for your region).
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