Cytosol&Particulate Fractionation Kit

Cat. No. CPS-002K **Lot. No.** (See product label)

SPECIFICATION

Product Overview	The Cytosol/Particulate Separation Kit provides an easy and convenient system for a rapid separation of cytosol and particulate fractions. Assaying cellular distributions of small moleculars or proteins, and other signal transduction pathways.
Usage	• Simple procedure; takes less than 1 hour • Fast and convenient • The new 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.
Storage	Store kit at -20centigrade. Briefly spin small vials prior to opening.
Kit Components	• Cell Suspension Buffer • Cytosol Releasing Buffer • Oil Layer • Particulate Layer
Preparation	1. After opening the kit, you may store Cell Suspension Buffer, Oil Layer and Particulate Layer at +4centigrade. Store Cytosol Releasing Buffer at –20centigrade. 2. Be sure to keep all kit components on ice at all times during the experiment. 3. The following protocol is described for fractionation of about 2 x 10^6 cells. If more cells are needed for fractionation, scale up the volume proportionally. 4. If desired, protease inhibitors can be added to the Cytosol Releasing Buffer to

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	prevent protein degradations.
Separation Protocol	1. Prepare Oil-Particulate Layers in a microcentrifuge tube: Add 40 µParticulate Layer into a microcentrifuge tube, 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 4centigrade. 3. Resuspend cells (~2 x 10^6 cells) in 40 µCell Suspension Buffer. 4. Add 40 µCytosol Releasing Buffer. Pipette up and down to mix well. 5. Apply the sample on top of the Oil-Particulate Layers prepared in Step 1. (Note: 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 (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 –70centigrade 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.

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