Cytosol/Particulate Rapid Separation Kit

(Catalog #K267-50; 50 separations; Store kit at –20˚C)

I. Introduction:

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. 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. Subcellular localization and analyses of factors interested can be performed accurately.

II. Kit Contents:

Component	K267-50 50 assays	Color Code	Part Number
Cell Suspension buffer	2 ml	Red	K267-50-1
Cytosol Releasing Buffer	2 ml	Green	K267-50-2
Oil Layer	25 ml	WM	K267-50-3
Particulate Layer	2 ml	Blue	K267-50-4

III. General Consideration and Reagent Preparation:

1. After opening the kit, you may store Cell Suspension Buffer, Oil Layer and Particulate Layer at +4˚C. Store Cytosol Releasing Buffer at –20˚C.
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 prevent protein degradations.

IV. Cytosol/Particulate Separation Protocol:

1. Prepare Oil-Particulate Layers in a microcentrifuge tube:

   Add 40 µl Particulate 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 4˚C.
3. Resuspend cells (~2 x 10^6 cells) in 40 µl Cell Suspension Buffer.
4. Add 40 µl 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. (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 –70˚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.

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