# Cytosol/Particulate Fractionation Kit

PromoKine

## Instruction Manual

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| **Catalog Number** | PK-CA577-K267 |
| **Description** | 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 Fractionation Kit physically separates cytosol from particulate compartments very rapidly through an oil layer - and thus the two fractions do not have any contact and cannot mix with each other. Using this method, contaminations can be avoided even for small molecules. Subcellular localization and analyses of interesting factors can be performed very accurately. |
| **Quantity** | 50 assays |
| **Kit Components** | Components — Quantity — Cap code<br>Cell Suspension Buffer — 2 ml — Red<br>Cytosol Releasing Buffer — 2 ml — Green<br>Oil Layer — 25 ml — WM<br>Particulate Layer — 2 ml — Blue |
| **Applications / Assay Protocol** | A. General Consideration and Reagent Preparation:<br>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.<br>Be sure to keep all kit components on ice at all times during the experiment.<br>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 accordingly.<br>If desired, protease inhibitors can be added to the Cytosol Releasing Buffer to prevent protein degradations.<br><br>B. Separation Protocol:<br>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.<br>2. Collect cells by centrifugation at 600 x g for 5 minutes at 4°C.<br>3. Resuspend cells (~2 x $10^6$ cells) in 40 µl Cell Suspension Buffer.<br>4. Add 40 µl Cytosol Releasing Buffer. Pipet up and down to mix well.<br>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 (at Step 4).<br>Note: The time cells interact with Cytosol Releasing Buffer is critical. 30 seconds seems to be optimal. Shorter incubation time may result in incomplete release of cytosol, whereas longer incubation time may result in contaminations.<br>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.<br>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.<br>8. Store both fractions at –70°C for further analyses. Generally, 30-40% proteins are in the cytosol fraction.<br>Note: If Oil Layer has diffused into the fractions, the fraction may be centrifuged again to remove oil.<br><br>Nuclear/Cytosol Fractionation Protocol:<br>A. General Consideration and Reagent Preparation:<br>After opening the kit, you may store buffers at +4°C or –20°C. Store Protease Inhibitor Cocktail and DTT at –20°C.<br>Add 250 µl DMSO to dissolve the 500X Protease Inhibitor Cocktail before use.<br>Before starting the procedure, prepare enough Nuclear Extraction Buffer Mix (NEB Mix) and Cytosol Extraction Buffer A Mix (CEB-A Mix) for your experiment: Add 2 µl Protease Inhibitor Cocktail and 1 µl DTT to each 1 ml of NEB and each 1 ml of CEB-A, individually. |

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| | Be sure to keep all buffers on ice at all times during the experiment. All centrifugation procedures should be performed at 4°C.<br>The following protocol is described for fractionation of up to 2 x $10^6$ cells. The procedure is also applicable for large-scale preparations (e.g., up to $10^9$ cells) by scaling up the volume.<br><br>B. Nuclear/Cytosol Fractionation Protocol:<br>1. Collect cells by centrifugation at 600 x g for 5 minutes at 4°C.<br>2. Add 0.2 ml CEB-A Mix containing DTT and Protease Inhibitors (prepared as in Section A). If using tissue samples, cut the tissue (100-200 mg) into small pieces, add ice cold PBS (1-2 ml), and homogenize in a tissue homogenizer. Pellet the cells by centrifugation at 500 x g for 2-3 minutes and remove the supernatant. Add 0.2 ml of the CEB-A Mix.<br>3. Vortex vigorously on the highest setting for 15 seconds to fully resuspend the cell pellet. Incubate the tube on ice for 10 minutes.<br>4. Add 11 µl of ice-cold Cytosol Extraction Buffer-B to the tube. Vortex 5 seconds on the highest setting. Incubate on ice for 1 minute.<br>5. Vortex 5 seconds on the highest setting. Centrifuge the tube for 5 minutes at maximal speed in a microcentrifuge (16,000 x g).<br>6. Immediately transfer the supernatant (cytoplasmic extract) fraction to a clean pre-chilled tube. Place the tube on ice.<br>7. Resuspend the pellet (contains nuclei) in 100 µl of ice-cold Nuclear Extraction Buffer Mix (prepared as in Section IIIA).<br>8. Vortex on the highest setting for 15 seconds. Return the sample to ice.<br>9. Repeat Step 8 every 10 minutes for a total of 40 minutes.<br>10. Centrifuge the tube at full speed (16,000 x g) in a microcentrifuge for 10 minutes.<br>11. Immediately transfer the supernatant (Nuclear extract) to a clean pre-chilled tube. Place on ice. Store extract at –80°C for future use.<br>Note: Nuclear extract prepared using the above procedure contains proteins in a concentration ~1 mg/ml. If higher concentration is desired, the nuclei can be resuspended in less volume of NEB-Mix (such as 20 µl) in Step 7. |
| **Intended Use** | For in vitro research use only. Not for diagnostic or therapeutic procedures. |
| **Storage & Stability** | Store kit at –20°C upon arrival. Store individual reagents as indicated on the respective labels. |

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