Apoptosis Cytosolic and Membrane Fraction Buffer Kit

Catalog: B108
Pack Size: Total Homogenate Buffer (THB)-B106 (100mL)
& Cytosolic Extraction Buffer (CEB)-B107 (60mL)

Description	
Description	For the preparation of cytosolic and membrane fractions from tissues or cells for use in the Apoptosis MAP assays.
Protocol: Cultured Cells	Keep all tubes (1.5 mL polypropylene tubes or equivalent), buffers and cell samples on ice during entire procedure. Prepare the buffers by adding protease inhibitors [Roche Diagnostics GmbH (Cat #11 836 153 001) or equivalent]. Once inhibitors are in solution, store the buffer at 4°C for up to 1 week or at -20°C for up to 3 months. **a.** Collect the cells ($5x10^6$ to $2x10^7$) by centrifugation for 5 minutes at 500xg. **b.** Wash the cells in 1-2ml of cold PBS. Centrifuge at 4°C for 5 minutes at 500xg. Remove supernatant. **c.** Freeze the pellet at -80°C for a minimum of 15 minutes. Remove and place on ice. **d.** Add 200µL of CEB to the pellet (or less for fewer cells); resuspend cells by pipetting up and down 10-15x then vortex for 5 seconds. **e.** Carefully place a pestle in one of the tubes containing the cells and buffer. Pass the pestle grinder [Fisher Scientific (Cat # 12-141-368) or equivalent] or Dounce homogenizer for ~ 20 times with a smooth twisting motion. **f.** Incubate the samples on ice for 15 minutes, quickly vortexing at each 5 minute interval. **g.** Centrifuge the homogenized samples at 4°C for 10 minutes at 10,000xg. **h.** Carefully remove the supernatant with a pipette and transfer to a new tube. Do not disturb the pellet. This supernatant is the cytosolic fraction. Measure the volume of cytosolic fraction removed, add an equal amount of THB then vortex. Store on ice while processing the membrane fractions. **i.** Wash the pellet from the cytosolic fraction by resuspending in 200µL of CEB and centrifuge at 4°C for 10 minutes at 10,000xg. **j.** Aspirate and discard the supernatant, taking care not to disturb the pellet. Resuspend the pellet in 200µL of THB. Be sure to completely break up pellet by mixing with pipette. Vortex for approximately 10 seconds and incubate sample on ice for 15 minutes, vortexing for 10 seconds at each 5 minute interval. **k.** Centrifuge the samples at 4°C for 10 minutes at 10,000xg. Carefully transfer supernatant to a new tube. This is the membrane fraction. **l.** Determine protein concentration of the samples using a 1:20 dilution in PBS [Pierce Coomassie Plus (Bradford) Assay Kit (Cat #23236) or equivalent] **m.** Process immediately or store samples at -80°C

Apoptosis Cytosolic and Membrane Fraction Buffer Kit

Catalog: B108
Pack Size: Total Homogenate Buffer (THB)-B106 (100mL)
& Cytosolic Extraction Buffer (CEB)-B107 (60mL)

Protocol: Tissue or Tumors	Keep all tubes(1.5 mL polypropylene tubes or equivalent), buffers and cell samples on ice during entire procedure. Prepare the buffers by adding protease inhibitors [Roche Diagnostics GmbH (Cat #11 836 153 001) or equivalent]. Once inhibitors are in solution, store the buffer at 4°C for up to 1 week or at -20°C for up to 3 months. **Part 1** **1. Tissue Sample with Precellys Homogenizer** **a.** Add tissue sample to a homogenization tube (Precellys Cat# KT03961-1-003.2, KT03961-1-009.2, or KT03961-1-002.2). Add 350 µL of CEB (500 µL for samples > 20 mg). **b.** Place tube into homogenizer at 4°C and shake at 5000 rpm for 30 seconds. Allow to settle for 30 seconds and repeat shaking at 5000 rpm for an additional 30 seconds. If large fragments are visible, homogenize for an additional 15 seconds. Remove any large fragments from the sample after this step. **2. Tissue Sample with Homogenizer** **a.** Add tissue sample to a tube. Add 100µl of CEB. Mince the tissue 10-15x using micro scissors. Add the remaining CEB to reach volume required (350 µL of CEB total; 500 µL for samples > 20 mg). **b.** Homogenize for approximately 5 seconds on medium power. Return to ice. If fragments are visible, homogenize for an additional 5 seconds. Remove any large fragments remaining after this step. **3. Tissue Sample with Pestle Grinder** **a.** Add 100µL of CEB to the tube containing the sample. Grind the tissue for approximately 10 seconds or until no large pieces remain. Add the remaining CEB to reach volume required (350 µL of CEB total; 500 µL for samples > 20 mg) remove any large fragments that remain. **Part 2** **c.** Incubate the processed samples on ice for 10 minutes. Transfer the sample to a new pre-chilled tube (minus beads and any pellet). **d.** Centrifuge the homogenized samples at 4°C for 10 minutes at 10,000xg. **e.** Carefully remove the supernatant with a pipette and transfer to a new tube. Do not disturb the pellet. This supernatant is the cytosolic fraction. Measure the volume of cytosolic fraction removed, add an equal amount of THB then vortex. Store on ice while processing the membrane fractions. **f.** Wash the pellet from the cytosolic fraction by resuspending in 750µL of CEB and centrifuge at 4°C for 10 minutes at 10,000xg. **g.** Aspirate and discard the supernatant, taking care not to disturb the pellet. Resuspend the pellet in THB with 350 µL of THB (500 µL for samples > 20 mg). Be sure to completely break up pellet by mixing with pipette. Vortex for approximately 10 seconds and incubate sample on ice for 15 minutes, vortexing for 10 seconds at each 5 minute interval. **h.** Centrifuge the samples at 4°C for 10 minutes at 10,000xg. Carefully transfer supernatant to a new tube. This is the membrane fraction. **i.** Determine protein concentration of the samples using a 1:20 dilution in PBS [Coomassie Plus (Bradford) Assay Kit (Cat #23236) or equivalent] **j.** Process immediately or store samples at -80°C
Shipping	Ambient
Storage	2-8°C
Expiration	See lot specific CoA

AMPERSAND BIOSCIENCES
3 Main St., Saranac Lake, NY 12983 | PHONE: (518) 891 0204 |