Apoptosis Total Homogenate Buffer

Catalog: B106
Pack Size: 100ml

Description	
Description	For the preparation of tissue or cells homogenates 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 homogenate -buffer 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 (volume can be adjusted for lower cell numbers) of prepared homogenate buffer to the pellet; resuspend cells by pipetting up and down 10-15x then vortex for 5 seconds. **e.** Keep the suspension on ice. **f.** Homogenize on ice using 20- 40 passes with a pestle grinder [Fisher Scientific (Cat # 12-141-368) or equivalent] or Dounce homogenizer. **g.** Transfer the sample to a new pre-chilled tube. 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. **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

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 30µL of prepared homogenate buffer per 1mg of tissue sample. **b.** Place homogenization tube (Precellys Cat# KT03961-1-003.2, KT03961-1-009.2, or KT03961-1-002.2) 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. Homogenized tissue samples should be free of large tissue fragments. 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 100µL of prepared homogenate buffer to the tube containing the sample. Mince the tissue 10-15x using micro scissors. Add the remaining lysis buffer (30µL per 1mg of sample) to the tube. **b.** Homogenize for approximately 5 seconds on medium power. Homogenized samples should be free of large tissue fragments. 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 prepared homogenate buffer to the tube containing the sample. Grind the tissue for approximately 10 seconds or until no large pieces remain. Add the remaining volume of homogenate buffer (30µL per 1mg of sample) and remove any large fragments that remain. **b.** Incubate the processed samples on ice for 10 minutes. **Part 2** **c.** Incubate the processed samples on ice for 10 minutes. **d.** Transfer the sample (minus beads and any pellet) to a new pre-chilled tube. 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. **f.** Determine protein concentration of the samples using a 1:20 dilution in PBS using the Pierce Coomassie Plus (Bradford) Assay Kit. **g.** Process immediately or store samples at -80°C
Shipping	Ambient
Storage	2-8°C
Expiration	See lot specific CoA