



Systems Virology

of Emerging Respiratory Viruses

PCL003.3P - Rapid Quenching Protocol for Cell Line Metabolomics Studies

(Modified on by Amy Sims and Tom Metz, March 2012)

Solutions Needed:

1. Rapid Quenching Solution
60% Methanol – Must use Thermo-Fisher Scientific A452-1 (1 liter bottle)
0.85% Ammonium Bicarbonate
qs with sterile H₂O

This solution is best store at -80C prior to use; however, precipitation of ammonium bicarbonate may occur at -80C. In this case, warm slightly and swirl to dissolve any precipitation prior to use.

2. Chloroform/Methanol Solution (Must be in a glass container)
2 volumes of Chloroform
1 volume of Methanol
Store at -80C or below prior to use
3. 150mM Ammonium Bicarbonate Solution
4. 8M Urea in 50 mM Bicarbonate Solution

Disposables/Equipment Needed:

1.5mL siliconized eppendorf tubes (Fisher part no. 02-681-331)
Speed vac

Procedure: (This procedure assumes that cells are in 6 well plates.)

1. Remove media from cells and add 3mL of -80C rapid quenching solution as rapidly as possible. Remove rapid quenching solution.
2. Repeat step 1 twice more.
3. Add 150uL of ice-cold 150mM ammonium bicarbonate solution and scrap cells to detach from plate. Collect cells and buffer into a 1.5mL siliconized eppendorf.
4. Add 0.6 mL -80C chloroform/methanol (a 4 fold excess).
5. Vortex sample for 10 seconds.
6. Let sample stand on ice for 5 minutes.
7. Vortex sample for 10 seconds.
8. Centrifuge at 13,000g for 10 minutes.
9. Remove upper phase to a new 1.5mL siliconized eppendorf.
10. Using a pipetman, go through the protein interphase, collect the lower phase from

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the bottom of the tube and place into a new 1.5mL siliconized eppendorf. Stop prior to mixing the remainder of the two phases.

11. Add 300uL 8Murea to protein interface, resuspend and freeze at -80C.
12. Evaporate both of the recovered phases to dryness in speedvac using a cycle that does not heat the samples. This will take ~2 h.
13. Freeze samples at -80C prior to shipping and ship samples to PMC Core on dry ice.
Note: infected samples must be safety tested following each experiment prior to shipping.

