Protocol for the preparation of dNTP samples from mammalian cells

*For the dNTP assay, prepare extracts from 2x10*6 cells using a protocol described below based on the cell type.*

Non-adherent cells

1) Count cells and prepare cell pellets with 2x10*6 cells.

2) Wash cell pellet with PBS, and remove as much PBS as possible.

3) Lysis the cell pellet by quickly resuspending the cells in ice cold 65% methanol (100ul per 1x10*6 cells).

4) Vigorously vortex samples for 2 min.

5) Completely lyse the cells by incubating at 95 ^{o}C for 3 min (make sure an epi tube lid lock is securely in place to prevent opening during the 95 ^{o}C incubation).

6) Chill on ice for 1 minute (to prevent burning your hands).

7) Centrifuge the tube for 3 min at 14K RPM and transfer the 65% methanol solution to a new label tube with identifiable numbers. Discard the tube with the pellet.

8) Speed vacuum dry the samples.

9) Ship the dNTP samples on dry ice to the following address below.

Adherent cells

1) Wash cell monolayer twice with PBS. (Always have an extra well for cell counting for each of the experimental groups).

2) Lysis the cell monolayer by quickly adding ice cold 65% methanol (100 ul per 1x10*6 cells). Scrape the cells off the plate, THOROUGHLY. Add another 100 ul of ice cold 65% methanol to wash the well to recover all the biomaterial. Place all the material into one tube for that sample. Have the tubes on ice while processing multiple samples.

3) Vigorously vortex samples for 2 min.

4) Completely lyse the cells by incubating at 95 ^{o}C for 3 min (make sure an epi tube lid lock is securely in place to prevent opening during the 95 ^{o}C incubation).

5) Chill on ice for 1 minute (to prevent burning your hands).

6) Centrifuge the tube for 3 min at 14K RPM and transfer the 65% methanol solution to a new label tube with identifiable numbers. Discard the tube with the pellet.

7) Speed vacuum dry the samples.

8) Ship the dNTP samples on dry ice to the following address below.

FedEx package to this address:

Dr. Baek Kim
c/o Caitlin Shepard
1760 Haygood Drive
HSRB, Room E450
Atlanta, GA 30322
Phone: (404) 727-1454
e-mail: baek.kim@emory.edu