Lazzara Lab
Janine Buonato
Updated by MJL June 18, 2017

Lysing adherent mammalian cells

Preparation of lysis time course:

If you are performing a time course experiment, plan it well beforehand. With multiple plates, leave enough time between plates so that you can not only treat the wells but also aspirate and lyse before the next plate has completed its time course at the end. It is prudent to leave ~2 min between plates. Here is an example:

00:60:00 stimulate ctrl 60 min well
00:58:00 stimulate kd 60 min well
00:45:00 stimulate ctrl 45 min well
00:43:00 stimulate kd 45 min well
00:30:00 stimulate ctrl 30 min well
00:28:00 stimulate kd 30 min well
00:15:00 stimulate ctrl 15 min well
00:13:00 stimulate kd 15 min well
00:05:00 stimulate ctrl 5 min well
00:03:00 stimulate kd 5 min well
00:00:00 lyse ctrl plate (all 6 wells)
-00:02:00 lyse kd plate (all 6 wells)

Materials:

- plated cells (6-well plates)
- lysis buffer
- rectangular and circular ice buckets with ice
- ice cold 1X PBS
- cell lifters/scrapers

Setup:

1. Label three sets of Eppendorf tubes. Use a different color for each set to avoid confusion.
   - crude lysates, label with a marker
   - clarified lysates, print labels on “Tough-Spots” using a template
   - BCA assay samples, label with a marker
2. Get two buckets of ice ready, one to hold your six well plates during lysis and one for LB and PBS tubes.
3. Mix up lysis buffer or IP lysis buffer, depending on what you will do with the samples. See separate protocols for preparation of these buffers. Note that lysis buffer can also be prepared during your time course depending on how much time you’ll have between time steps. Mixing takes very little time, ~5 min for the lysis buffer recipes we use.
4. Perform your experiment. Follow your planned time course closely. Note that you can complete your final stimulation or whatever the experiment calls for out on the bench to be sure that lysis occurs on schedule.
5. TIME SENSITIVE - When the timer hits 0 knock the six well plate into the ice, aspirate each well.
6. TIME SENSITIVE - Next rinse each well with about 2 mL of ice cold PBS and FULLY aspirate. Prop plate at angle, aspirate once, then go back for a second pass to get as much out as quickly as possible.
7. TIME SENSITIVE – Lastly, add 100 µL LB per well. This breaks up all the cells and freezes all catalytic activity so the rest of the steps are not time sensitive.
8. Using a different cell lifter for each well, vigorously scrape each well for 10-15 sec. Before finishing, scrape the blade of the cell lifter on the side of the well to remove any lysate stuck to it.
9. Prop the plate against the side of the bucket to allow liquid to pool in well corners. Pipette each well up and down a few times, taking up at the bottom and releasing the lysate over the plate to rinse it a few times.
10. Pipette ALL liquid out of each well into the tubes labeled for “crude lysate.”
11. Vortex each tube briefly and spin crude lysates at 4ºC, max rpm for 10 min. Orient each tube similarly so that if you cannot see the cellular debris after it’s spun down you know where it should be.
12. Make BCA assay sample tubes by adding 10 µL of sample to 90 µL H_2O. Vortex each tube briefly, and store at 4ºC.
13. Collect supernatant from each tube and put into the printed label clarified lysate tubes. Store at -80ºC.