Institut für HIV Forschung
SOP #14-00 **(April 2015)**

Chromium 51 Release Assay with Peptide

Feed the EBV cell line 1:1 the day *before* the assay -- this will lower spontaneous release of targets.

Separate radioactive waste very carefully from regular waste. Monitor your work area after each use of this or any other radioactive isotope. Record usage of ^{51}Cr every time. When the vial reaches half its total volume, it is a good idea to verify the actual amount of ^{51}Cr in the tube -- sometimes there is less ^{51}Cr in the vial because somebody forgot to record when they removed some.

1. Select the appropriate targets (EBV line) expressing the restricting HLA class I allele and transfer the required number of cells into a 15 ml conical tube. Transfer 1-1.5 million cells per target into a 15 ml conical tube, depending on how many targets you will need for the assay; plan the assay in advance so that you will know how many cells you need.
2. Spin cells down for 10 min at 1500 rpm.
3. Aspirate supernatant to about 200 µl and add peptide at 10 µg/ml FC.
4. Add 50 µCi of ^{51}Cr per target, then resuspend cells with the same pipet tip you used to add the chromium. Pipet tips containing chromium should be discarded in the empty media/PBS bottle used for chromium pipet tip waste.
5. Incubate cells for 1 hour at 37° C.
6. In the meantime, count the effectors and resuspend them at the proper concentration, which is most likely 1M/ml or .5M/ml for an Effector:Target ratio of 10:1 or 5:1. Place these tubes in the incubator until you are ready to make the effector plate.
7. Take out a 96-well rounded bottom plate and write all the necessary information on it. This will help you put the correct cells into the correct wells. Make sure to put markings on the plate itself as well as the lid to avoid confusion with the lids from multiple plates.

After the hour is up, wash the targets three times with 12 ml cold R10 at 1000 rpm for 7 min

8. in a cold centrifuge.
9. Count cells and resuspend at 1 x 10^5 cells/ml (.1M/ml). Place on ice and prepare the effector plate.
10. When the effector plate is ready, add 100 µl targets to each well using a multichannel pipettor or a repeater pipet. Remember to make a Max plate with triple wells. Do it on a separate plate to avoid splashes.
11. Incubate plates for 4 hours in the incubator.
12. After 4 hours, harvest 30 µl of supernatant onto a Luma plate using a multichannel pipettor.
13. Add 30 µl of 5% Triton per well and let the Luma plate dry overnight at room temperature in a hood.
14. Add 30 µl of expose to the chromium plate. Aspirate all liquid waste into the chromium vacuum flask and then dispose of the plate as chromium dry waste.
15. Measure the Luma plate the next morning on the Wallac counter.

Preparation of Targets for Chromium assay

Note: media and centrifuges should be kept at 4 ° C.

1. Count viable and non-viable cells from vaccinia infected target cells following protocol. Greater than 70% viability is needed for a successful assay.
2. Remove 1.5 million cells and place in a 15 ml conical centrifuge tube, one for each target.
3. Spin at 1500 rpm for 10 min.
4. Aspirate all but 200 µl of the supernatant.
5. Add 50 µCi of ^{51}Cr. (As of 2002, 10 µl of ^{51}Cr = 10 µCi.)
6. Incubate for 45 min at 37 °C.
7. Wash 3 times with 12 ml of cold R10, centrifuging at 4 °C for 7 minutes at 1000 rpm.
8. After the final wash, add 3 ml of R10. Resuspend gently and remove a small aliquot (less than 200 µl) for counting.
9. Count viable and nonviable targets.
10. Dilute targets to the correct final concentration of 1 x 10^5 cells/ml (.1M/ml) and keep them on ice until you are ready to add them to the plate.
11. Prepare the effector plate with the correct peptides and effectors as the chosen ratio. Take care to avoid contaminating adjacent wells when adding peptides to the plate.
12. Add targets last to the plate and incubate them in the incubator for 4 hours, 6 hours if you are doing the assay on fresh PBMCs.
13. Harvest 30µl of supernatant for Luma plates. Remember to add 30µl of 5% triton to each well of the Luma plate. Dry Luma plates overnight in the hood before counting the next day.
14. When you put your plates on Wallac to be counted make sure that you have written the file number that the assay will be saved under.
15. Dispose your radioactive plates appropriately. It is your responsibility to transfer them to the lower shelf and bag them all and tag them for proper disposal when full. If you need instruction on this, just ask!
16. Clean and dispose radioactive waste after **each** assay! There is no radioactive storage in the tissue culture room at all!

Radioactive waste disposal after ^{51}Cr assays

1. All Cr51-contaminated waste must be decontaminated with first. Aspirate the liquid into the chromium flask, then put the solid waste into a plastic bag (we use empty bags from centrifuge tubes). These are the strongest bags we have available. They are to be sealed and marked "Cr51".
2. Extra large bags to line the barrels are kept in the chromium hood, which is labeled as the chromium storage area.
3. When the barrel is full, the large bag should be sealed and 'tagged' using yellow radiation waste disposal tags. Activity is usually less than 200 mCi for solids and liquids must be written on the jar, so that the person sealing them knows exactly what amount to write on the disposal tag. Jars with liquid must be double bagged and then tagged.
4. Solid and liquid radioactive waste is picked up every Thursday. We do not have to call them because they come automatically. (Need to change procedure based on WRAIR)
5. All supplies for radiation disposal such as jugs and bags can be obtained free from the Radiation Safety Office, down the hall from the PARC administrative office on our floor
6. Chromium waste MUST be removed from TC room after each assay .

Other concerns

Be aware that Cr51 is a gamma emitter with a half life of 27.7 days and a max energy of 0.32MeV. This means that lead shielding of 1.7mm will block 50% of the gamma rays. A Standard NaI or Geiger counter will easily detect the source. The relatively short half life means that the source will need to be replenished regularly, so if it's been awhile since the last assay you might need to purchase more.