Chemotaxis Assay

by Melissa Swope, 3/12/2000

Purpose

The purpose of a chemotaxis assay is to determine whether your protein or small molecule of interest has chemotactic activity on a specific cell type. Chemotaxis is the ability of a protein to direct the migration of a specific cell. This assay is based on the premise of creating a gradient of the chemotactic agent and allowing cells to migrate through a membrane towards the chemotactic agent. If the agent is not chemotactic for the cell, then the majority of the cells will remain on the membrane. If the agent is chemotactic, then the cells will migrate through the membrane and settle on the bottom of the well of the chemotaxis plate.

Using CEM cells

Media and culture conditions:

RPMI 1640

10% FBS (not heat-inactivated)

5 μg/ml gentamycin (1:100 of the lab stock)

Split 2x per week 1:10. Culture in 10% CO_2, 37ºC.

Materials

- Neuroprobe 96-well chemotaxis plate 5 μm pore size CAT# ChemoTx# 101-5
- Favorite chemokine of choice (100 μg/ml)
- Fixation media: 2.5% FBS, 3% formaldehyde, in L15 media
- Small glass FACS tubes
- 96-well round bottom plate
- 12 well or 8 well multi-channel pipette
- Multi-channel pipette dish to hold the fixation media

Procedure

1. Count cells. Add 10 μl of cells to 90 μl of trypan blue in one well of a 96-well plate. Take 10 μl and load onto a hemacytometer. Count the outer 4 squares and the middle square. Divide this number by 5 to get the number of cells per square. One square is equal to $1x10^4$ cells/ml of a 1:10 dilution. Therefore, multiply the number by 10 and this gives you the number of cells/ml of culture.

   Example: there are 25 cells in 5 squares = 5 cells/square. This is the equivalent of $5x10^4$ cells/ml of a 1:10 dilution. Multiply $5x10^4$ time 10 = $5x10^5$ cells/ml of the original culture.
2. Spin the cells down at 1400 RPM, 4 minutes.
3. Remove the supernatant and resuspend the cell to $1x10^7$ cells/ml in fresh culture media. Leave the cells on ice until you are ready to use them.
4. Make 1:10 serial dilutions of chemokine for the bottom chamber of the chemotaxis plate. You will need 30 μl per well. I generally use the plate in columns rather than rows. Therefore, I usually make 7 serial dilutions (10 μg/ml down to 0.01 ng/ml) and use the last well for a buffer control.

5. Load the chemotaxis plate. The chemotaxis plate is a 96-well plate from Neuroprobe consisting of a bottom plate, a membrane, and a cover. You need to add enough chemokine to the bottom well so that it looks as if it is almost over the top of the well. When you place the membrane on top of the wells, this slight excess of media will form a seal between the membrane and plate. Depending on your specific pipette, this volume amounts to 28-30 µl. Make sure you add the media such that there are no small bubbles formed. Bubbles will inhibit migration of cells across the membrane.
6. In addition, to one column of the plate you will need to make a percent input standard curve. This is for later analysis to determine the percent of chemotaxis for your samples. Add 25 µl of culture media to each well of one column. Take 25 µl of the cell suspension and pipet up and down in the first well of the column. This well is now the equivalent of 50% of the cell input. Continue to dilute 1:1 all the way down the column until you reach the last well. These 1:1 dilutions result in a standard curve with the following percent input values: 50, 25, 12.5, 6.25, 3.13, 1.56, 0.78, and 0.39. An example of a typical chemotaxis assay is attached to this protocol.
7. Place the membrane over the bottom plate. There are 4 pegs (one on each corner) that the membrane slides on to. Make sure the membrane is firmly seated on the pegs. You can tell because all of the wells with chemokine should form a seal with the membrane (the well will look darker when sealed properly).
8. Add 25 µl of the cell suspension to the top of each well. There is a hydrophobic coating surrounding the membrane for each well which allows the cell droplet to form a round sphere. Be sure that you don't touch the membrane with the tip of your pipette.
9. Put the cover over the cell droplets and carefully place in a 37ºC tissue culture incubator for 2 hours and 15 minutes.
10. After chemotaxis, gently remove the plate from the incubator. Pry off the membrane using the thin end of a metal spatula. It is important to remove the membrane in one fluid motion. Don't move the membrane up and down during the process of removal because this causes a small amount of suction resulting in drawing some cells through the membrane. You can tell if this has happened by looking at the wells after removal of the membrane. If there are cells floating rather than lying flat on the bottom, then you have accidentally drawn some cells through the membrane.
11. Using a multi-channel pipette, add 50 µl of fixation media to each well and pipet up and down. Transfer the entire volume (~80 µl) to a glass FACS tube which has been placed in a 96-well round bottom plate.
12. Count samples for 15 seconds each on the FACScan. Use the percent input wells as a standard curve to determine the percent chemotaxis for each sample.

Expected Results

Depending on the chemokine, you should expect a bell shaped curve with the maximal chemotaxis from 1-10 ng/ml and decreasing chemotaxis at high concentrations (1-10 µg/ml).

References

ChemoTx® System Protocol provided by NeuroProbe Inc.