STANDARD OPERATING PROCEDURE Transwell Migration Assay

Author: Jonathan Pribila File name: SOPtranswell UPDATED: 04/10/01 10:39 AM

HAZARDS

Human T cells and human blood products (human serum albumin).

Avoid ingestion, inhalation, or skin contact with human blood products to avoid contact with blood-borne pathogens.

PROTECTION

Lab coat Gloves Absorbant bench paper

WASTE

All human waste must be disposed of according to Occupational Safety and Environmental Health Protection guidelines, including:

SOLID waste in autoclavable trash Pipets (NOT including pasteur pipets) Tubes LIQUID waste must be bleached (1/10 vol) prior to sewering Glass trap connected to aspiration set-up containing bleach

SHARPS waste (Red biohazard sharps container) for razors and pasteur pipets

PROCEDURE

Materials and Equipment

Costar Transwell Migration Plates (#3421) 9.6 um Beads (Interfacial Dynamics Corp. #1 10000) Purified ligand (e.g., fibronectin, ICAM-1, etc.) Phosphate buffered saline (PBS) without Ca²⁺ and Mg²⁺ Syringe pipet (Micromatic #5720, Popper and Sons, Inc.) 50 ml polystyrene centrifuge tubes (e.g., Corning #430290) IEC Centra-7R refrigerated centrifuge (or equivalent) Complete RPMI medium Repeating pipet (Nichiryo Co. Model #8100 or equivalent) 37°C water bath Inverted light microscope

Solutions

Serum Starvation Media 500 mls RPMI

STANDARD OPERATING PROCEDURE FOR TRANSWELL MIGRATION ASSAYS SHIMIZU LAB DATE MADE: 4/11/01 DATE PRINTED: 05/04/01 Page 1 X:\protocols\SOPtranswell.doc 5 mls L-glutamine
2.5 mls Pen./Strep
5 mls 1M Hepes
5 grams BSA
pH to 7.0 before each experiment and filter sterilize
Solution should be a yellow orange when it is at the correct pH.

FN

Human Fibronectin (20 ug/ml) Dilute 1 ml stock (1mg/ml) to 50 mls in PBS without Ca++/ Mg++

BSA (20 ug/ml) Dissolve 0.2 grams of BSA in 100 mls PBS without Ca++/ Mg++ Filter sterilize

1X Trypsin

Dilute 10 mls of 10X trypsin (Gibco-BRL #15090-046)in 90 mls of PBS without CA++/Mg++

1mM EDTA

Add 0.37224g of EDTA (Fisher #BP120-500) to 1L of PBS without CA++/Mg++

FACS Buffer + 10% BCS

500 mls HBSS + 0.2% Sodium Azide 50 mls BCS FACS Buffer + 10% BCS

Procedure COATING THE PLATES WITH FN OR BSA

• To the outer wells marked with an X add 500 ul of FN or BSA

X	X	Х	Х	Х	Х	Х
Х	Х	Х	Х	Х	Х	Х

- Transfer the transwell insert from the inner wells to the outer wells.
- Place the insert in at an angle to prevent air bubbles from forming
- If bubbles do form then touch with a sterile yellow pipette tip or touch gently to the side of the plate and reinsert.

STANDARD OPERATING PROCEDURE FOR TRANSWELL MIGRATION ASSAYS SHIMIZU LAB DATE MADE: 4/11/01 DATE PRINTED: 05/04/01 Page 2 X:\protocols\SOPtranswell.doc

- Using a repeat pipetter, add 100 ul of either FN or BSA to the top of the well and incubate overnight at 4 C.
- Alternatively, the plates can also be coated the day of the experiment for 2 hrs at 37C.

DRYING THE PLATES

- Remove insert and aspirate off the FN or BSA from the insert using a glass pasteur pipette with a yellow tip on the end. Try not to touch the filters so that you do not disrupt the FN and do not poke a hole in the filter.
- Also aspitate off the FN or BSA from the bottom of the plate.
- Place the insert back in the same well in which it was coated.
- The insert generally take about 30 minutes to completely dry. When dry they become slightly cloudy.

SERUM STARVATION

- Count cells
- Spin down cells at 1500 RPM for 5 minutes at room temperature and pour off supernatent
- Resuspend to $1.0*10^6$ cells per ml in serum starvation media and incubate for 4 hours.
- If the cells are transient transfectants then:
 - Underlay the cells with 10 mls of ficol and spin at 2000 rpm for 20 minutes with the brake off
 - Using a 10 mls pipette harvest the live cells from the interface and transfer to a new 50 mls conical
 - Wash 2X with PBS without Ca++/Mg2++, spinning the cells down at 1500 RPM for 5 minutes at room temperature between washes.
- Count the cells
- Resuspend at 0.5e6/100ul or else 5e6/ml in serum starvation media

MIGRATION ASSAY

- Dilute SDF to the desired concentration
 - SDF-1 α Stock is 100 ug/ml
- To the lower chambers of the inner set of wells add 0.5 ml of migration media -/+ SDF-1a
- Transfer the transwell insert from the outer wells to the inner wells.
- Place the insert in at an angle to prevent air bubbles from forming
- If bubbles do form, then touch with a sterile yellow pipette tip or touch gently to the side of the plate and reinsert.
- Example is shown below:

| 0 ug/ml |
|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|
| 30 ng/ml
SDF-1α |
| | | | | | |

• Add 0.50e6 cells/well in 100 ul of serum starvation media to the upper chamber of the appropriate wells as well as 3-5 additional FACS tube containing 1 mls of FACS Buffer + 10% BCS. These additional

FACS tubes will serve as premigrated controls. Using a repeat pipetter works the best. Place at 4C while the other cells are migrating.

- CAREFULLY put plate in 37C incubator, do not mix up the upper and lower chambers!
- Incubate for 2.5 hours.

CELL HARVESTING AND FACS ANALYSIS

- Remove inserts from the well and carefully remove media from upper wells using clean plastic pipette tip on end of aspirator (try not to directly touch the filter if you can).
- Remove the media from the lower well and transfer it to a FACS tube. Do not pool the cells from multiple wells. They all go into separate FACS tubes. Place a FACS tube rack in an ice bucket to keep the harvested cells cool.
- Add 500ul mixture of 25 mls 1mMEDTA and 25 mls 1XTrypsin to each lower well. To do this, hold filter carefully over well and gently rinse dislodged cells from underside into well by adding EDTA/trypsin solution to lower edge of insert so that media flows across undersurface and into the well. BE VERY CAREFUL NOT TO SHOOT MEDIA CONTAINING DISLODGED CELLS INTO OPEN PORTIONS OF TRANSWELL INSERTS THAT GO INTO UPPER CHAMBER.
- Place the insert back in the well witht the Trypsin/EDTA solution and allow plates to incubate for 3-5' at RT.
- Remove Trypsin/EDTA from the lower chamber and transfer it to the appropriate FACS tube.
- Add an additional 500 ul of Trypsin/EDTA solution in the same manner as described above.
- Place the insert back in the well and incubate the plates at 37C for 10 minutes.
- Remove Trypsin/EDTA from the lower chamber and transfer it to the appropriate FACS tube. Pipette up and down several time to dislodge any adherent cells.
- Add 500 ul of FACS Buffer + 10% BCS as described above.
- Brush the FACS Buffer + 10% BCS against the side of the well so that any liquid clining to the bottom falls into the well
- Place the transwell insert into the outer well
- Pipette up and down several time to dislodge any adherent cells and remove the FACS Buffer + 10% BCS and place it into the appropriate FACS tube.
- Spin the cells down at 1500 rpm for 5 minutes at 4C (Don't forget to also spin down the premigrated controls and the FACS controls described below).
- Dump off supernatents and add 200 ul of FACS Buffer + 10% BCS.

PREPARATION OF THE BEADS

- Squirt some of the stock beads into an eppendorf tube and store this tube in your 4C box.
- Dilute the beads from this eppendorf tube 1:200 in FACS Buffer + 10% BCS.
- Count beads on hemocytometer to determine the number of beads per ml.
- Add 50 ul to each tube with a repeat pipetter.

PREPARATION OF PI

- Dilute stock of PI (2mg/ml) 1:50 in FACS buffer
- Add 25 ul a couple of minutes before FACSing tube.

FACS CONTROLS

There are four FACS controls

- 1. Untransfected cells
- 2. Transfected cells (done with any remaining transfected cells)
- 3. Transfected cells + Beads (done with any remaining transfected cells)
- 4. Beads

STANDARD OPERATING PROCEDURE FOR TRANSWELL MIGRATION ASSAYS SHIMIZU LAB DATE MADE: 4/11/01 DATE PRINTED: 05/04/01 Page 4 X:\protocols\SOPtranswell.doc

STANDARD OPERATING PROCEDURE FOR TRANSWELL MIGRATION ASSAYS SHIMIZU LAB DATE MADE: 4/11/01 DATE PRINTED: 05/04/01 Page 5 X:\protocols\SOPtranswell.doc