MAGNETIC SORTING OF TRANSFECTED CELLS USING THE MACSELECT SYSTEM

Materials:

MACSelect K ^{k}.II plasmid
OctoMACS magnet
MS separation columns
PBE:
PBS supplemented with 0.5% BSA and 5 mM EDTA
PBE must be degassed before use

Procedure:

1. Transfect cells by method of choice on 100 mm plate.

   Cotransfect your plasmid of interest along with the MACSelect K ^{k}.II plasmid (encodes mouse MHC class I H-2K^{k} protein with truncated cytoplasmic domain to avoid signal transduction). Use a 1:1 ratio.

   OR

   Clone your gene of interest into the MACSelect K ^{k}.II plasmid and transfect into your cell line.

2. Incubate 24-48 hours (determine optimal time for your cell type- best expression occurs after one cell doubling).

3. Harvest less than or equal to 1 X 10^{7} transfected cells (out of a maximum number of 2 X 10^{8} total cells):

   a. Wash cells with PBS, removing all dead cells.
   b. Add 500 µl trypsin and allow to trypsinize in incubator.
   c. Stop trypsinization by adding 100 µl of 100% serum.
      To avoid clumping of HeLa cells: Add 5 ml of PBE to trypsinized cells and resuspend. Check under the microscope that there are no clumps. Spin down the cells and remove the supernatant. Add 600 µl PBE and resuspend.
   d. Add 80 µl of MACSelect K ^{k} Microbeads (binds to cells expressing the MACSelect K ^{k}.II- encoded protein).
   e. Rock plate gently by hand to disperse. Incubate 15 min at RT, rocking twice more during the incubation.
   f. Add PBE to adjust the final volume to 2 mls (add 1320 µl) and resuspend cells completely. Verify cells are not clumped. If they are, separate by passing several times through an 18 gauge needle or a 1 mL pipette tip.

4. Magnetic separation:

    a. Attach OctoMACS Separation Unit to the MACS MultiStand.
    b. Put MS column on the OctoMACS Separation Unit. Place a waste tube under the column.
    c. Apply 500 µl PBE to column and let run through into collection tube.
    d. Save 100 µl aliquot of cells (for "pre-column" control), and apply the rest to the column 500 µl at a time. Resuspend each aliquot well before applying to the column.
    e. Wash the column 4 times with 500 µl PBE.
    f. Place column in collection tube.
    g. Add 1 ml PBE, and flush out cells with plunger into collection tube.

5. Fluorescence detection:

    a. Take 100 µl of cells in PBE (from the "pre-column" and the "post-column" samples) and add 10 µl of H-2K^k FITC.
    b. Incubate in the dark at 4C.
    c. Wash with 1 ml PBE, spin, and remove supernatant.
    d. Resuspend pellet without additional buffer.
    e. Transfer to glass slide and visualize by microscopy.
    f. Determine percent enrichment.
    g. Cells can be enriched again on a fresh column to give higher percent enrichment if necessary.

Reference:

Miltenyi Biotech, www.miltenyibiotec.com

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