Assay of cell adhesion molecules in transfected cells grown in suspension

Carlos Ibez
Department of Neuroscience, Karolinska Institute, S-17177 Stockholm, Sweden
Fernanda Ledda
Department of Neuroscience, Karolinska Institute, S-17177 Stockholm, Sweden

Method Article

Keywords: cell adhesion, Jurkat cells

Posted Date: March 8th, 2007

DOI: https://doi.org/10.1038/nprot.2007.164

Abstract

Introduction

This is an assay to test for cell adhesion activities in transmembrane proteins and mutants thereof.

Reagents

This procedure requires a DNA construct of the molecule\(s) of interest in a plasmid vector for expression in mammalian cells, EGFP and Ds-Red plasmids, Fugen6 transfection reagent from Roche, and Jurkat cells, a human T-cell line that grows in suspension.

Procedure

1. Day 1. Prepare an exponential culture of Jurkat cells in complete medium \(RPMI, 10% fetal bovine serum, gentamycin). 2. Day 2. Seed 2 ml from a $5x10^5$ cells/ml Jurkat cell suspension in 12 well-plates \(i.e. $1x10^6$ cells per well). Each DNA construct to be tested \(or controls as appropriate) will require 2 wells. 3. Prepare transfection mixes as follows: i) combine 4.5 µg of each expression construct \(or empty vector control) with 0.5 µg of EGFP plasmid; ii) in separate reactions combine another 4.5 µg of each expression construct \(or controls as appropriate) with 0.5 µg of Ds-Red plasmid; iii) prepare Fugene6 dilution by combining 12 µl of Fugene6 \(per transfected well) with 160 µl of serum- and antibiotic-free RPMI medium, leave to activate at room temperature for 5 min; iv) add 170 µl of Fugene6 dilution to each DNA mix, leave 15 min at room temperature. 4. Add each transfection mix drop-wise to a well with Jurkat cells. You will end up with green \(EGFP) and red \(Ds-Red) series of all constructs to be tested. Incubate in CO_2 incubator overnight. 5. Day 3. Prepare a 48-well plate with 100 µl of serum-free RPMI \(but containing antibiotics) in each well. Prepare 4 times as many wells as adhesion assays \(for quadruplicate determinations). Ligands or other substances capable of modifying or altering cell adhesion may also be added here. Allow additional 4 wells for each new condition to be tested. 6. Combine 100 µl of cell suspensions from the green and red series and add to each of quadruplicate wells in 48 well-plate containing plain medium or ligands. Mix well and incubate in CO_2 incubator for 2 days. 7. Day 5. Resuspend cells by carefully shaking the plate side-wise, and spin for 1 min at low speed to pellet the cells to the bottom of the wells. For best results, the cells should lie at the bottom uniformly covering the whole well. 8. Count manually under epifluorescence illumination \(FITC and Rhodamine channels) the number of green and red cells, and the proportion of each forming part of cell aggregates containing green and red cells. Cell adhesion can be expressed as the percentage of green \(or red) cells present in mixed cell clusters. 9. Alternatively, photographs can be taken in an inverted epifluorescence microscope. We have developed an automated procedure for OpenLab software with which we typically photograph three horizontal rows of 8 fields each across the well diameter at 10X magnification using a motorized Axiovert 200 microscope. Using the photographs, green and red cells and overlapping cell clusters \(yellow) can be quantified off-line, either manually of through a second OpenLab automation that counts

everything automatically and saves the results as a tab-delimited document. OpenLab automations are available upon request.