

Cell separation (1) THP-1

Introduction

THP-1 is a human monocytic cell line. This cell differentiates into macrophage by PMA (Phorbol 12-Myristate 13-Acetate) stimulation, and is used in macrophage studies. We performed model experiments of cell separation using THP-1.

Summary

We evaluated the cell separation ability of commercially available Streptavidin immobilized magnetic beads using the biotin-labeled THP-1. FG beads have a higher collection rate than other beads in our study.

Result 1 (Column)

We compared FG beads with other commercially available magnetic beads by use of Magnetic column.

We confirmed FG beads have a superior ability for the collection of live THP-1. This verifies FG beads are useful for the cell separation application.

Beads	Collection rate (%)	Standard deviation
FG beads	35.8	3.3
A	9.7	4.6
B	6.9	4.5
C	18.7	9.7
D	5.9	3.5

*MACS Column MS and Mini MACS Separator (Miltenyi Biotec)

Result 2 (Stand)

We compared FG beads with competitor A and B by use of Magnetic stand.

We confirmed FG beads have a superior ability for the collection of live THP-1.

Beads	Collection rate (%)	Survival rate (%)	Survival rate (%) after 24h	Proliferation rate (%) after 24h
Non(untreated)	-	97	98	190
FG beads	61.3	95	99	190
A	1.0	86	91	200
B	4.8	98	96	140

*Magnet Stand (cat#TA4899N12)

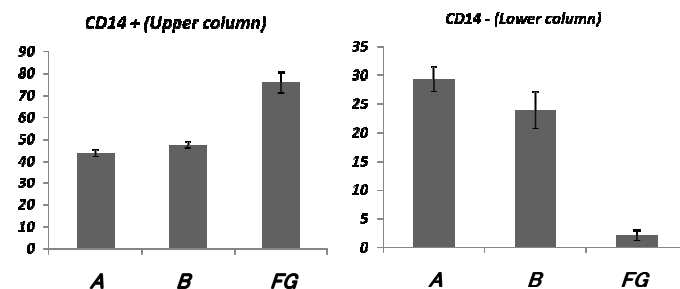
Result 3 (Flow cytometry)

We compared FG beads with competitor A and B by use of Magnetic column.

Evaluation was performed by the flow cytometry.

'Upper column' is the number of cells which were trapped in the column.

'Lower column' is the number of cells which passed through the column.



Result 4 (Methods)

We compared antibody binding methods.

'cells': Cells were labeled with biotinylated antibody first, then biotin-labeled cells were separated by Streptavidin beads.

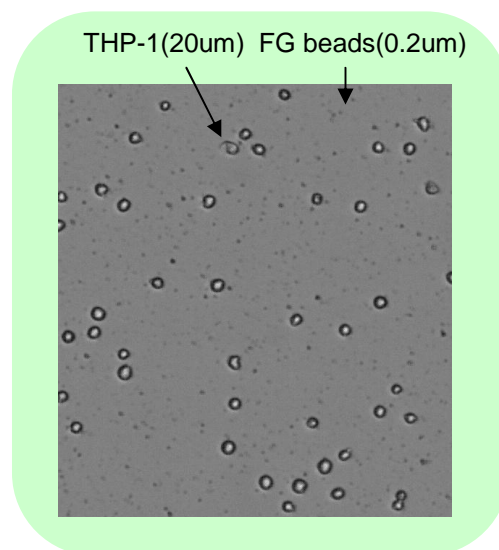
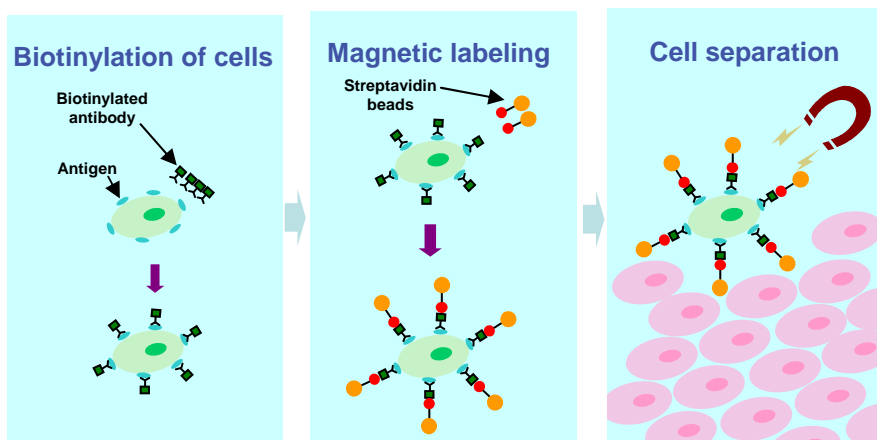
'beads': Biotinylated antibodies were bound to Streptavidin beads first, then cells were separated by antibody-immobilized beads.

We experienced better results with the biotinylation of cells.

Antibody	Collection rate (%)	Survival rate (%)	Survival rate (%) after 24h	Proliferation rate (%) after 24h
Non(untreated)	-	95	97	170
cells	50.0	94	97	180
beads	5.0	91	95	140

*Magnet Stand (cat#TA4899N12)

Experimental Information



Materials and method

Materials

1. Streptavidin beads
(cat#TAS8848N1170 and competitor A, B, C and D)
2. THP-1 Cell line
3. Biotinylated CD14 antibody (SM3009B, Acris Antibodies)
4. Labeling Buffer (PBS(pH7.2), 2mM EDTA, 0.5% BSA)
5. Separation Buffer (PBS(pH7.2), 2mM EDTA, 0.5% BSA)
6. Cell counting Kit 8 (Dojin Chemical)
7. Trypan Blue (Bio-Rad)

Apparatus

1. Magnetic Stand : Magnet Stand (cat#TA4899N12)
2. Column : MACS Column MS (Miltenyi Biotec)
Mini MACS Separator (Miltenyi Biotec)



Magnet Stand (cat#TA4899N12)



MACS Column MS and Mini MACS Separator
(Miltenyi Biotec)

Methods 1 (Biotinylation of THP-1 cells)

1. **Add biotinylated antibody to THP-1 Cells**
Add 500ul of 1/250 diluted antibody to 10^7 THP-1 cells in Labeling Buffer.
2. **Reaction**
Mix for 10min at 4°C.
3. **Wash and Store**
Remove the unreacted antibody by centrifugation.
Wash with 500ul of Labeling buffer 2 times.
Add 90 ul of Labeling buffer to the precipitated biotin-labeled THP-1 cells.

Methods 2 (Magnetic-labeling of THP-1 cells)

1. **Add beads to THP-1 cells**
Add 0.1mg (10ul) of Streptavidin beads to 90ul of biotin-labeled THP-1 cells.
2. **Reaction**
Mix for 15min at 4°C.

Methods 3 (Wash and separation of beads-bound cells)

Column

1. Apply to column

Apply cell suspension to MACS Column and Separator.

2. Wash and Recover

Wash with 500ul of Separation Buffer 3 times.

If column is used, remove the column from the Separator and then collect beads-bound cells from the column with injector.

Transfer to culture medium.

Stand

Note: When Magnet Stand is used, take care not to suction cells when removing supernatant, because cell pellet may not be completely intact after magnetic separation.

Methods 4 (Evaluation)

Count cell viability with Cell Counting Kit 8 (Result 1), or Trypan Blue (Result 2).