

Isolation of Transfected Cells

Rapid and easy magnetic selection of transfected cells.

Require no reagent preparation or special equipment to see the beads.

An extremely simple, fast and inexpensive method.

More sensitive than flow cytometry for transfected cell isolation (1).

Introduction

Isolation of successfully transfected cells using Dynabeads® products provide many advantages over conventional isolation methods including speed and sensitivity. In transient gene transfer, the expression of the recombinant gene product can be assessed 60 hours after transfection instead of the normal three week period using drug selection. One can easily isolate transfectants present at low frequencies. The system's speed makes it ideal for isolating cells expressing a transient gene.

Dynabeads® Epoxy, Tosylactivated and Secondary coated products can be used with your own antibody (Ab) to a cell surface molecule (CSM) to isolate the transfected cells. Alternatively, the Dynabeads® Primary coated products can be used to mark, identify and isolate cells co-transfected with common lymphocyte markers (2).

Applications

Retroviral Expression Cloning

A highly efficient selection strategy using mAb and Ab-coated Dynabeads® (Sheep anti-Mouse IgG) to screen the retroviral expression library. Cells were cultured in the presence of the magnetic beads, with no apparent perturbation in cell growth (3).

HLA class-II Gene Expression in HeLa Cells

Combines the strong mammalian expression vector CDM8, with an efficient transfection protocol for immunomagnetic selection (Dynabeads® Goat anti-Mouse IgG incubated with mAb). The technique has been used to obtain transformants expressing the three human class-II MHC molecules at high levels individually and in combination. Cells expressing surface markers at a high level in a stable fashion can be isolated directly from transfected populations by repeated immunomagnetic selection over 2-3 weeks (1).

Transiently Transfected Cells

A plasmid encoding for a neural cell-specific surface marker is co-transfected into mammalian cells with the gene of interest. After uptake and expression of these two plasmids, the transfected cells are immunoadsorbed to Dynabeads® M-280 Streptavidin pre-coated with biotinylated mAb for the surface marker (axonin-1). After magnetic isolation, the cell population can be enriched more than 7-fold for a co-transfected reporter. Selected cells are used for further cultivation or analysis (4).

Electrophysiological, Biochemical and Imaging Studies

Human Embryonic Kidney (HEK) 293 cells were transiently transfected with different ratios of GluR1 and GluR2(R) cDNAs. The subunit specific cDNAs on separate CDM-8 plasmids were co-transfected with the cDNA for the lymphocyte surface antigen CD8 in a 4:1 ratio. Transiently transfected cells were isolated using immunomagnetic Dynabeads® pre-coated with a monoclonal human anti-CD8 antibody and used in electrophysiological, biochemical and imaging studies (2).



Fig. 1. Human CD4+ cell bound to two Dynabeads® CD4. (Dr H Ping Ting-Beall).

Plasmid rescue

A plasmid-based transgenic mouse model used for studying *in vivo* mutations. A high degree of efficiency recovers millions of plasmid copies in one experiment. About 100,000 transformants are routinely obtained from 1 µg genomic DNA (about 30% of theoretical maximum efficiency, assuming a copy no. of 20 and a transformation efficiency of 1×10^{10}).

Reduced costs compared with *lacI* and *lacZ* bacteriophage- λ models. Plasmid containing the *lacZ* reporter gene are excised from genomic DNA by HindIII restriction enzyme digestion and separated from total genomic DNA using *lacI* repressor proteins coupled to Dynabeads® M-280 Streptavidin. After circularisation by ligation, plasmids are electrotransferred into *E.coli C lacZ*, *galE* host cells (5).

Materials & Methods

Dynal Magnetic Particle Concentrator (DynaL MPC®). Buffers: (phosphate-buffered saline (PBS) /0.1% bovine serum albumin (BSA), Tris-buffered saline (TBS) /2 mM EDTA. Cell culture medium with, e.g. fetal calf serum (FCS). Transfection assay. Expression vectors (to quantify the efficiency of cell selection). Cell fixation and Cytostaining.

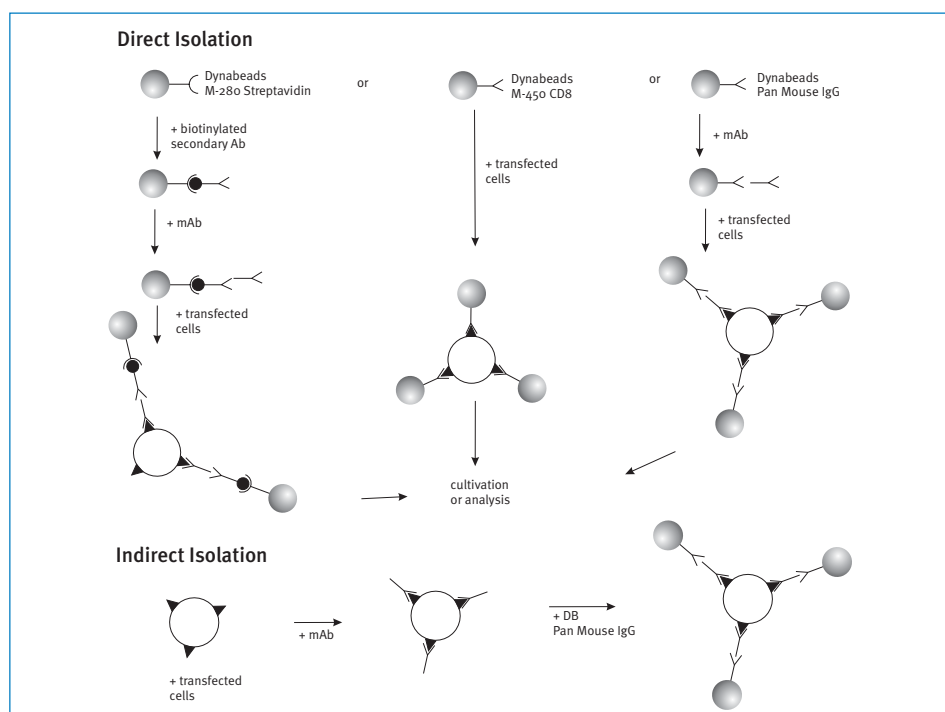


Fig. 2: Different isolation techniques to isolate transfected cells.

Ordering Information

Dynal Product	Prod. No.
Dynabeads® M-450 Epoxy	140.11
Dynabeads® Pan Mouse IgG	110.41/42
Dynabeads® Sheep anti-Mouse IgG	110.31
Dynabeads® Goat anti-Mouse IgG	110.33
Dynabeads® CD8	111.47
Dynabeads® M-280 Streptavidin	112.05/06
DynaL MPC®-S	120.20
DynaL MPC®-L	120.21

Subsidiaries: Denmark, Sweden, Norway and Iceland: Dynal Biotech ASA Tel: +47 22 06 10 00, Fax: +47 22 50 70 15, USA, Canada: Dynal Biotech Inc. 1 800 558 4511, UK, Ireland: Dynal Biotech Ltd 0 800 731 9037, Germany, Belgium, Austria, Switzerland and Holland: Dynal Biotech GmbH +49 40 36 15 73 0, Australia & NZ: Dynal Biotech PTY Ltd. (Aust) 1800 623 453, (NZ) 0800 448 246, France, Belgium and Switzerland: Dynal Biotech S.A. +33 3 44 23 45 95, China: Dynal Biotech (Beijing) Ltd: 8610 67806708, Japan: Nihon Dynal K.K.: 81 3 3593 7861.

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Protocol

(Indirect technique)

1. Transfect cells with DNA and plate for 1-2 days to express surface antigen.
2. Incubate cells with saturating amounts of mAb for 30-90 min. on ice with intermittent mixing. Wash cells twice in PBS w/ 2% FCS to remove unbound Ab and resuspend at $2-4 \times 10^7$ cells/ml.
3. Add secondary Ab (or streptavidin)-coated Dynabeads® (e.g. Pan Mouse IgG) to cells and incubate for 20-60 min. at 2-8°C on a mixer. Generally, 4 Dynabeads® / target cell are recommended using both indirect and direct techniques, given a concentration of at least 1×10^7 Dynabeads®/ml.
4. Capture bead-bound cells by applying the tube to a magnet for 1-2 min. Supernatant containing unbound cells is removed whilst bead-bound cells remain on the tube wall.
5. Remove the tube from the magnet and wash cells with PBS w/ 2% FCS. Place tube back on magnet and repeat step 4. Wash cells 4x before resuspending in culture medium.
6. Culture cells in the presence of Dynabeads® (which do not interfere with growth/ attachment of most cell types) for a few days to expand cell numbers.
7. Selection procedure is repeated twice to enrich positive clones to homogeneity.

References

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