Introduction

Human embryonic stem cells can be differentiated into neural, mesenchymal and hematopoetic stem cells. This product is intended for the magnetic depletion of SSEA-4^{+} undifferentiated embryonic stem cells from a differentiated cell population to increase purity. Isolated SSEA-4 negative cells are bead- and antibody free and are suitable for any downstream application. The products can also be used to isolate the undifferentiated SSEA-4 positive cells, however, SSEA-4^{+} cells will bind to Depletion MyOne SA Dynabeads® and removal of beads after cel lysis is recommend prior to analysis.

Principle of Isolation

SSEA-4 antibodies are biotinylated and bind to the non-differentiated SSEA-4^{+} cells in the mixed cell population. Depletion MyOne SA Dynabeads® are added and will bind to the antibody labeled cells during a short incubation. The bead-bound cells are subsequently separated on a magnet. The remaining untouched and bead-free cells in the supernatant can be used for any application. If undifferentiated SSEA-4+ cell will be used, gene and protein analysis can be performed after lysis of the cells. Place the lysed cells in the magnet to remove beads prior to analysis.

Downstream Applications

Differentiated stem cells (NSC, MSC, or HSC) depleted of SSEA-4^{+} cells can be analyzed in flow cytometry and used in downstream applications such as cell therapy/adoptive transfer, gene expression profiling, DNA methylation studies, immunohistology, protein expression analysis, further passaging/differentiation and drug discovery. The SSEA-4 positive cells are isolated in the bead-containing fraction and the undifferentiated SSEA-4^{+} embryonic stem cells can be further analyzed after cell lysis for gene- and protein analysis.

Additional Requirements

- Isolation Buffer: Ca^{2+} and Mg^{2+} free phosphate buffered saline (PBS) From Gibco (cat.no. 14190-094) supplemented with 0.1 % BSA (Cat. no. P2046) and 2 mM EDTA (Cat. no. 15575020).
- Mixer allowing both mixing and tilting.
- Magnet (DynaMag™): See www.invitrogen.com/magnets for magnet recommendations.

Critical Notes

- Use a mixer that provides tilting and rotation of the tubes to ensure that Dynabeads® do not settle at the bottom of the tube.
- This product should NOT be used with MPCTM-1.
- Never use less than the recommended volume of Dynabeads®.
- Carefully follow the recommended pipetting volumes and incubation times.
- Avoid air bubbles during pipetting.

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Ordering Information

Sku	Product Catalog	Size	Price	Quantity
15575-020	UltraPure™ 0.5M EDTA, pH 8.0	4 × 100 ml	62.00 USD	

Add to Cart

Protocol

Dynabeads® Washing procedure

1. Resuspend the Dynabeads® in the vial.
2. Transfer the desired volume of Dynabeads® to a tube (see Table 1).
3. Add the same volume of Isolation Buffer, or at least 1 ml, and mix.
4. Place the tube in a magnet for 1 min and discard the supernatant.
5. Remove the tube from the magnet and resuspend the washed Dynabeads® in the same volume of Isolation Buffer as the initial volume of Dynabeads® (step 2).

Preparations

- Prepare the Isolation Buffer.
- Prepare a single cells suspension of the cells to a concentration of 1×10^7 cells/ml Isolation Buffer.

	Volumes per $\leq 5 \times 10^6$ Cells	**Volumes per 5×10^7 Cells**	**Volumes per 5×10^8 Cells**
Tube size	1.5 - 5 ml	15 ml	50 ml
Cell volume (step 1)	500 µl	500 µl	5 ml
SSEA-4 Antibody (step 2)	25 µl	250 µl	2.5 ml
Depletion MyOne	50 µl	500 µl	5 ml

SA Dynabeads® (step 5)			
Volume Buffer added (step 7)	1 ml	2 ml	20 ml

Isolation Procedure

This protocol is based on 5×10^6 cells and is scalable from 5×10^6 to 5×10^8 cells according to Table 1.

Table 1. Volumes for isolation

Note that optimal bead depletion occurs at a certain concentration of bead/ ml, thus, when working with cell numbers lower than 5×10^6, never use less than 50 µl of beads. If fewer beads are used, reduced depletion can be observed.

The starting number of cells for this protocol is 5×10^6 (see preparation above for details).

1. Transfer 500 µl (5×10^6) cells in Isolation Buffer to a tube.
2. Add 25 µl SSEA-4 Antibody and mix well.
3. Incubate 15 min at 18-25 ℃.
4. Add 1 ml Isolation Buffer and spin cells for 8 min at 300 × g.
5. Remove the supernatant and resuspend the cell-pellet in 500 µl Isolation buffer, add 50 µl of pre-washed Depletion MyOne SA Dynabeads®.
6. Mix well and incubate for 15 min at 18-25 ℃ with gentle tilting and rotation.
7. Add 1 ml of Isolation Buffer (if the volume from step 5 is greater than 1 ml, add the 1 ml Isolation Buffer after step 8).
8. Resuspend the bead-bound cells by pipetting > 10 times with a pipette with a narrow tip opening, (e.g. a 1000 µl pipette tip or a 5 ml serological pipette).
9. Place the tube in the magnet for 2 min.
10. Transfer the supernatant, containing the bead-free SSEA-4 negative cells, to a new tube.

11. Repeat step 9-10 to remove residual beads.

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General Information

Manufactured by Invitrogen Dynal® AS. Invitrogen Dynal® AS complies with the Quality System Standards ISO 9001:2000 and ISO 13485:2003.

Description of Materials

Depletion MyOne SA Dynabeads® are uniform, superparamagnetic beads (1.0 µm diameter) coated with streptavidin (SA). Supplied at 10 mg beads per ml in phosphate buffered saline (PBS), pH 7.4, containing 0.1% bovine serum albumin (BSA) and 0.02% sodium azide. SSEA-4 Antibody is a biotinylated monoclonal mouse antibody supplied in phosphate buffered saline (PBS), pH 7.4, containing 0.02% sodium azide.

Storage/Stability

This product is stable until the expiry date stated on the label when stored unopened at 2-8 ℃. Store opened vials at 2-8 ℃ and avoid bacterial contamination. Keep Dynabeads® in liquid suspension during storage and all handling steps, as drying will result in reduced performance. Resuspend well before use.

Technical Support

Please contact Invitrogen Dynal® for further technical information. Certificate of Analysis (CoA) is available upon request. The latest revision of the package insert/ instruction for use is available on www.invitrogen.com.

Warnings And Limitations

This product is for research use only. Not for animal or human therapeutic or diagnostic use. Follow appropriate laboratory guidelines. This product contains 0.02% sodium azide as a preservative, which is cytotoxic.
Avoid pipetting by mouth!

Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. When disposing through plumbing drains, flush with large volumes of water to prevent azide build up. Material Safety Data Sheet (MSDS) is available athttp://www.invitrogen.com.