VERITAS

VERITAS INFORMATION

未分化 iPS/ES 細胞の除去

Dynabeads™ 磁気ビーズを用いたネガティブセレクションの応用例

ヒト iPS/ES 細胞から分化させた細胞には、未分化であるヒト iPS/ES 細胞が残存し、将来腫瘍化する可能性があり、再生医療に応用する際の大きな問題となっています。
この未分化であるヒト iPS/ES 細胞を簡便に除去 (Depletion) する方法が多く検討されています。
その一方で以前より、均一な粒径を持つ Invitrogen™ Dynabeads™ 磁気ビーズを用いて胸腺中の未成熟な細胞 ($CD4^-CD8^-$ 細胞) を簡単に純度良く分離するなど、細胞分離で多くのアプリケーションが開発されています。
今回その一例として、分化誘導後の細胞集団から $SSEA\text{-}4^+$ 未分化 ES 細胞を除去することで、細胞の純度を向上させるアプリケーションを紹介します。

- **分化した細胞を高純度、高収率、高生存率で回収します**
- **純化された分化後の細胞は、ビーズと抗体が付いていないため、様々な下流のアプリケーションに使用可能です**
- **ヒト iPS/ES 細胞のコンタミネーションをなくし、下流アプリケーションへの影響を防ぎます**

DEPTHLETION OF SSEA-4+ HUMAN EMBRYONAL STEM CELLS USING DYNABEADS

Pauline Lieu[1], Ying Liu[1], Steve Spusta[2], Anette Kullmann[3], Xianmin Zeng[2], Berit Reed[3], John Chesnut[1], Karoline W. Schjetne[3]
[1] Invitrogen Corporate, Life Technologies, Carlsbad, California, US
[2] Buck Institute of Age Research, Novato, California, US
[3] Invitrogen Dynal, Life Technologies, Oslo, Norway

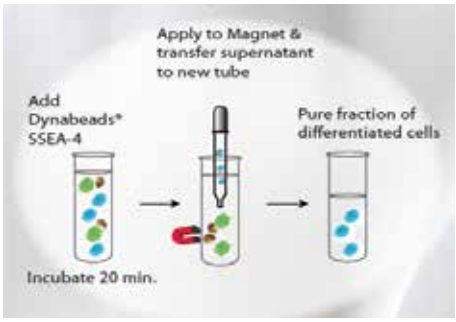
AIM: Develop a protocol for gentle depletion of undifferentiated human embryonal stem cells (hESCs)

Background

Human embryonic stem cells (hESC) research has become one of the most exciting and fastest growing areas in cell biology today and has an enormous potential for clinical applications and in drug discovery. Since embryonic stem cells have unlimited proliferative capacity and can differentiate into multiple cell types, they provide an inexhaustible source of precursors and differentiated cells for many therapeutic applications. Today, scientists have successfully used various methods to differentiate human embryonic stem cells into many different cell types, such as neurons and cardiomocytes. One of the challenges is to obtain a pure and homogenous population of target cells for drug screening or clinical applications.

Materials and Methods

Human embryonal stem cells (hESCs) were differentiated for 16 days into neuronal precursor cells using NSC medium. Cells were detached from the culture flask wall and analyzed for SSEA-4 expression. After differentiation, some cells still expressed SSEA-4 indicating that undifferentiated hESC are present (Fig 1a). Dynabeads® SSEA-4 were added to the cell suspension and placed on a magnet after a short incubation. The bead-free cells were transferred to a new tube and analyzed by flow cytometry showing >99% depletion of undifferentiated $SSEA\text{-}4^+$ cells (Fig 1b). Also, stainings of Oct4 (Fig 2) and nestin (Fig 3) were performed on the differentiated NSC demonstrating that the depletion protocol efficiently removed $Oct4^+$ cells (ESC) leaving a pure nestin positive (NSC) cell population. To show that the differentiated cells were unaffected by the depletion procedure, the cells were further differentiated into motor neurons and glial precursors. The morphology and specific expression of beta tubulin and A2B5 markers of the differentiated cells are visualized in fig 4.



Dynabeads® SSEA-4 remove hESC from the differentiated NSC populations

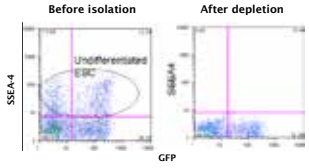


Fig 1: Human ESC stem cells (BG01) differentiated into NSC were stained with anti-SSEA-4 antibody pre- and post depletion using Dynabeads® SSEA-4 prior to analysis by flow cytometer.

Oct4 positive cells are removed after depletion of SSEA-4 depletion

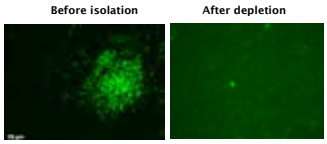


Fig 2: Oct4 positive cells (green) were detected in the differentiated NSC population before depletion (left), demonstrating that undifferentiated ESC were still present. After depletion of $SSEA\text{-}4^+$ cells using only background staining is observed (right).

Neural stem cells express nestin after SSEA-4 depletion

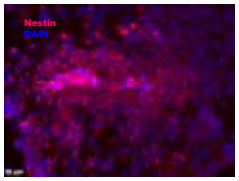


Fig 3: Differentiated NSC were stained with anti-nestin antibodies (pink) after depletion of SSEA-4 positive cells.

Neural stem cells depleted of $SSEA\text{-}4^+$ cells differentiate into motor neurons and glial precursor cells

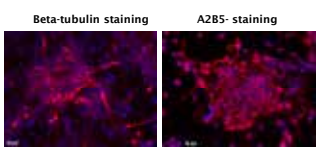


Fig 4: NSC depleted of $SSEA\text{-}4^+$ cells where further differentiated into motor neurons and glial precursors prior to staining with beta actin to identify neural precursor and anti-A2B5 mab to identify oligodendrocytes.

Ordering information	Cat.no	Application
Dynabeads® SSEA-4	111.60D	Depletion of undifferentiated $SSEA\text{-}4^+$ hESCs
DynaMagTM-2	123.21D	Holds 2 ml tubes
DynaMagTM-15	123.01D	Holds 5 ml and 15 ml tubes
Anti-SSEA-4 antibody	41-4000	Detection of SSEA-4+ cells
Anti-A2B5 antibody	41-1000	Detection of A2B5+ cells
StemPro® hESC SFM	A1000701	Culture of hESCs

CONCLUSIONS

- **Dynabeads® SSEA-4** efficiently deplete undifferentiated $SSEA\text{-}4^+$ hESCs
- SSEA-4 depleted neural stem cells further differentiate into neural precursor cells such as motor neurons and glial precursor cells
- Isolation is tube-based, fast and gentle to your cells

先の例ではビオチン化した抗 SSEA-4 抗体と Invitrogen™ Dynabeads™ MyOne Streptavidin を組み合わせて用いておりましたが、他にもビオチン化抗体と組み合わせることで、任意のマーカーを発現している細胞を除去することが可能です。

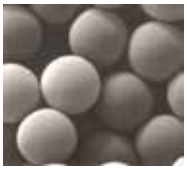
例として Nanog、Oct3/4、TRA-1-60、SSEA-3、SSEA-5 など

また、ビオチン化抗体と Dynabeads Streptavidin 磁気ビーズを用いなくても、お手持ちの抗体 (標識、未標識問わず) と 2 次抗体結合 Dynabeads を組み合わせることであらゆる種類の細胞分離に対応します。ターゲット細胞の表面マーカーに対する 1 次抗体をご用意するだけで、高純度の細胞分離や除去 (Depletion) が可能です。

蛍光標識された 1 次抗体をご利用いただければ、除去 (Depletion) 後、純度をフローサイトメトリーで確認し、もし不必要な細胞がまだ混在していたならば、磁気ビーズの処理を繰り返すことで、より純度の高い細胞を得ることが可能です。

Dynabeads 磁気ビーズとは

Dynabeads 磁気ビーズは可磁化物質（γ Fe_2O_3 と Fe_3O_4）が一様に分布した高分子ポリマーを機能性ポリマーで覆った、粒子径が均一なビーズです。この表面に種々の抗体を結合したビーズや、ある種の蛋白あるいはヌクレオチドなどを結合したビーズは、強力な磁石 (DynaMag) を利用することにより、細胞の分離ならびに蛋白や核酸などの分離・精製をきわめて簡便な操作で、確実かつ速やかに行うことができ、これまでの多くのアプリケーションで利用されています。



未分化 iPS/ES 細胞の除去に利用可能な Dynabeads 磁気ビーズ製品

製品コード	製品名	梱包単位
DB11031	Dynabeads M-450 Sheep anti-Mouse IgG	5 mL(2x10^9cells 用)
DB11033	Dynabeads M-450 Goat anti-Mouse IgG	5 mL(2x10^9cells 用)
DB11035	Dynabeads M-450 Sheep anti-Rat IgG	5 mL(2x10^9cells 用)
DB11039	Dynabeads M-450 Rat anti-Mouse IgM	5 mL(2x10^9cells 用)
DB11041	Dynabeads Pan Mouse IgG	5 mL(2x10^9cells 用)
DB11042	Dynabeads Pan Mouse IgG	5 x 5 mL(10x10^9cells 用)
DB11201	Dynabeads M-280 Sheep anti-Mouse IgG	2 mL
DB11202	Dynabeads M-280 Sheep anti-Mouse IgG	10 mL
DB11203	Dynabeads M-280 Sheep anti-Rabbit IgG	2 mL
DB11204	Dynabeads M-280 Sheep anti-Rabbit IgG	10 mL
DB11205	Dynabeads M-280 Streptavidin	2 mL
DB11206	Dynabeads M-280 Streptavidin	10 mL
DB65001	Dynabeads MyOne Streptavidin C1	2 mL
DB65002	Dynabeads MyOne Streptavidin C1	10 mL
DB65305	Dynabeads M-270 Streptavidin	2 mL
DB65306	Dynabeads M-270 Streptavidin	10 mL
DB65601	Dynabeads MyOne Streptavidin T1	2 mL
DB65602	Dynabeads MyOne Streptavidin T1	10 mL
DB65801	Dynabeads Streptavidin Trial Kit	1 mL x 4

日本総代理店

株式会社
ベリタス
〒105-0013 東京都港区浜松町1丁目10-14
住友東新橋ビル3号館5階
TEL.03-5776-0078(代) FAX.03-5776-0076
E-mail: veritas@veritastk.co.jp
http://www.veritastk.co.jp/