

Total Protein – Murine Embryonic Stem Cell Line D3

CATALOG NUMBER: CBA-305

STORAGE: -80°C; avoid freeze/thaw

QUANTITY AND CONCENTRATION: 500 µg at 1.0 mg/mL in NP-40 Solubilization Buffer

SHELF LIFE: 6 months from date of receipt under proper storage conditions

Background

Embryonic stem (ES) cells are continuous proliferating stem cell lines of embryonic origin first isolated from the inner cell mass (ICM). Two distinguishing features of these ES cells are their ability to be maintained indefinitely in an undifferentiated state and their potential to develop into any cell within the body.

ES-D3 was first isolated from day 4 129/Sv blastocysts in 1985 by Doetschmann et al. When injected into himeric blastocysts, ES-D3 contributed to the germ line. Alkaline phosphatase is routinely used as a marker of undifferentiated pluripotent stem cells. ES-D3 cells propagated on a feeder layer of irradiated STO mouse fibroblasts in the presence of recombinant mouse LIF are positive for alkaline phosphatase activity. Spontaneous differentiation of embryoid body cells can be induced by leukemia inhibitory factor withdrawal.

Quality Control

1. Monitor ES alkaline phosphatase activity in a control D3 plate to ensure the undifferentiated stage of D3 cells before lysis step.
2. Visualize the protein pattern on SDS-PAGE by Coomassie blue staining.
3. Analyze β -Actin expression level by Western blot.

Methods

1. Grow undifferentiated D3 cells on gelatin-coated dishes in the presence of LIF.
2. Before the cell lysis step, examine ES alkaline phosphatase activity in a control plate using StemTAG™ Alkaline Phosphatase Staining Kit (Cat. #CBA-300).
3. Wash cells twice with ice cold 1X PBS.
4. Add 1 mL of NP-40 Solubilization Buffer (20 mM Tris, pH 7.5, 150 mM NaCl, 1 mM MgCl₂, 1% deoxycholate, 5mM EDTA, 1% NP-40, 1 mM Sodium orthovanadate and a cocktail of protease inhibitors) to each 10cm plate and incubate on ice for 10 minutes.
5. Centrifuge at 12000 rpm for 10 minutes at 4°C.
6. Remove supernatant for protein assay and discard the pellet.

Example of Results

The following figures demonstrate typical results. One should use the data below for reference only. This data should not be used to interpret actual results.

4X Field

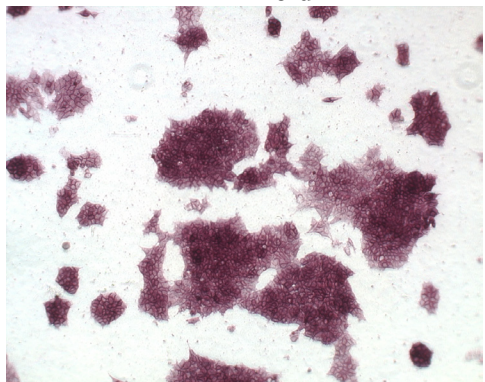


Figure 1. Alkaline Phosphatase Activity of Murine ES-D3 Cells. AP activity on undifferentiated cells was determined using the StemTAG™ Alkaline Phosphatase Staining Kit (Cat. #CBA-300).

References

1. Wobus AM, Holzhausen H, Jäkel P et al. (1984) *Exp Cell Res* 152:212–219.
2. Thomson JA, Itskovitz-Eldor J, Shapiro SS et al. (1998) *Science* 282:1145–1147.
3. Smith AG, Nichols J, Robertson M et al. (1992) *Dev Biol* 151:339–351.
4. Reubinoff BE, Pera MF, Fong CY et al. (2000) *Nat Biotechnol* 18:399–404.

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Contact Information

Cell Biolabs, Inc.
7758 Arjons Drive
San Diego, CA 92126
Worldwide: +1 858-271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com

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