# ES/iPS Differentiation Monitoring Kit - Mouse Endoderm
# Technical Manual

*Technical Manual (Japanese version) is available at http://www.dojindo.co.jp/manual/es02.pdf*

## General Information

The embryonic stem cells (ES cells) or induced pluripotent stem cells (iPS cells) are differentiated to various cells through the three germ layers: endoderm, mesoderm and ectoderm. One of the secretory proteins in a culture medium was found to be a marker of the conversion level of ES or iPS to endodermal cells. This secretory protein has a good correlation with Cxcr4 and E-cadherin which are commonly used proteins as markers of endoderm differentiation. Amount of this protein in the cell culture supernatant determined by ELISA, can be used to monitor the efficiency of differentiation of endodermal cells from ES/iPS cells. Since this kit is designed for 96-well microplate format, it is suitable for multiple sample measurements such as a screening with inducers of differentiation for ES/iPS cells.
This product was developed by a collaborative work between Institute of Molecular Embryology and Genetics, Kumamoto University and Dojindo Laboratories.

## Kit Contents

| | | | |
|---|---|---|---|
| - Coated 96-well Strip Plate | x 1 | - Storage Buffer | 0.5 ml x 1 |
| - Standard | x 1 | - Substrate Solution | 10 ml x 1 |
| - Enzyme-labeled Antibody | x 1 | - Plate Seal | x 3 |
| - Washing Buffer | x 1 | | |

## Storage Condition

Store at 0-5 °C.

## Required Equipment and Materials

- Microplate reader
- Single and multichannel pipettes
- Paper towel
- 1.5 ml tube
- Sulfuric acid

## Precaution

Centrifuge the tube briefly before opening the cap; ingredients may be attached on the wall or the cap of the tube.

## Preparation of Solutions

- Washing Buffer
  Dissolve the contents in the Washing Buffer packet with 1000 ml of deionized or distilled water.
  Store the Washing Buffer Solution at room temperature.
- Stop Solution
  Prepare 0.2 mol/l sulfuric acid solution with deionized or distilled water.
- Standard Stock Solution
  Add 30 µl of the Storage Buffer in the Standard tube (blue cap) and dissolve by gentle pipetting.
  *After dissolution, store at -20 °C and use within one month.
- Enzyme-labeled Antibody Stock Solution
  Add 30 µl of the Storage Buffer in the Enzyme-labeled Antibody tube (red cap) and dissolve by gentle pipetting.
  *After dissolution, store at -20 °C and use within one month.

## General Protocol

1) Take the plate out from the bag, and remove all the strips from the frame (photo A).
   *Before opening the bag, bring back to the ambient temperature.

2) Remove necessary number of strips from the seal and put them back to the frame (photo B and C). Keep the remaining strips in the tightly closed bag and store in a refrigerator (photo D).
   *If a strip comes off from the seal, press the strip tightly against to the seal or use a new plate seal to cover the strips.

3) Dilute the Standard Stock Solution 200-fold with the Washing Buffer in a 1.5 ml tube to prepare a 50 ng/ml of the Standard Solution. Prepare the following Standard Solutions by serial dilution using the Washing Buffer (Fig 1).
   *Standard Solutions: 25, 12.5, 6.25 and 0 ng/ml
   *Since the Standard Solution diluted with the Washing Buffer is not stable, prepare the necessary amount of Standard Solutions immediately before use.

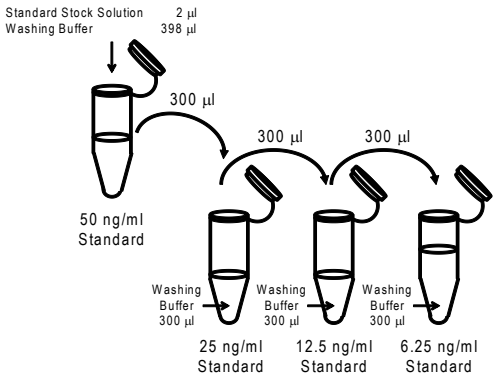
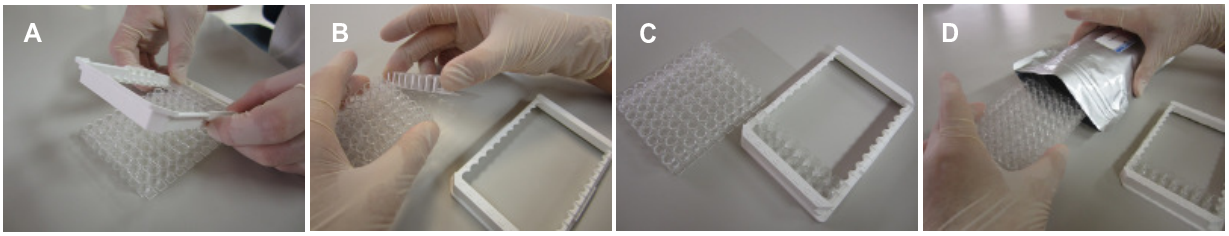



**Fig 1. Procedure of Standard Solution preparation (n=2)**



## General Protocol

4) Add 100 µl Standard Solution or the samples (cell culture supernatant) to each well and incubate for 1 hour at room temperature.
[1]*In the case of duplicate measurement, use two strips for four samples. By using multiple strips, more samples can be measured simultaneously (Fig 2).
[2]*If the absorbance of samples is over the range, dilute the samples to an appropriate concentration with the Washing Buffer.

5) Dilute the Enzyme-labeled Antibody Solution 1000-fold with the Washing Buffer to prepare Enzyme-labeled Antibody working solution.

6) Discard the solution, and wash the wells with 250 µl Washing Buffer three times. Tap the plate several times against paper towels to remove the buffer in each well. Add 100 µl Enzyme-labeled Antibody working solution to each well, then incubate for 1 hour at room temperature.

7) Discard the solution, and wash the wells with 250 µl Washing Buffer five times. Tap the plate several times against paper towels to remove the buffer in each well. Add 100 µl Substrate Solution to each well, and incubate for 10-15 minutes at room temperature.

| | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| A | 0 ng/ml Standard | | Sample E | |
| B | 6.25 ng/ml Standard | | Sample F | |
| C | 12.5 ng/ml Standard | | Sample G | |
| D | 25 ng/ml Standard | | Sample H | |
| E | Sample A | | Sample I | |
| F | Sample B | | Sample J | |
| G | Sample C | | Sample K | |
| H | Sample D | | Sample L | |

**Fig 2. Example of Standard Solution and Sample arrangement (n=2)**

8) Add 100 µl Stop Solution to each well, and measure the absorbance at 450 nm by microplate reader. Calculate the amount of the marker protein in the samples using the calibration curve obtained from the Standard Solution.

## Usage Example

**- Monitoring endoderm differentiation derived from mouse ES cells -**

Mouse ES cells were seeded to the concentration of 5.0 x $10^3$ cell/well on a 24-well plate and cultured in differentiation medium containing 10 ng/ml Activin A and 5 ng/ml bFGF. The medium was changed in every 48 hours. The 100 µl of the supernatant was analyzed by this ELISA kit. The amount of secreted marker protein was compared with the percentage of differentiation to endodermal cells analyzed by flow cytometry. The amount of secreted marker protein in the supernatant was found to correlate well with the amount of endoderm differentiation under different culturing conditions.

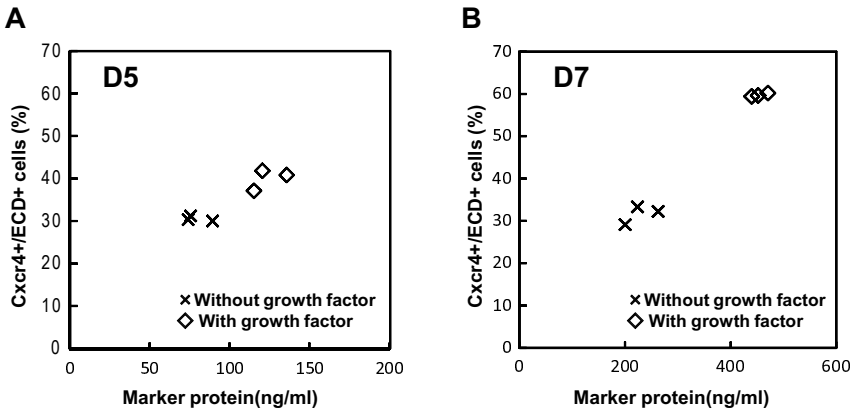



**Fig 3. The correlation of marker protein in supernatant with endodermal cells at day 5 (A) and day 7 (B).**
*Endodermal cell: Cxcr4 and E-Cadherin double positive cell

## Notes

The amount of the marker protein may vary depending on the differentiation method or cell density. In order to determine the differentiation ratio by using this kit, please prepare the correlation graph with the ELISA data and immunostaining or flowcytometry data.

## Reference

H. Iwashita, N. Shiraki, D. Sakano, T. Ikegami, M. Shiga, K. Kume, S. Kume., *PloS ONE.*, **2013**, *8*(5): e64291.

*If you need more infomation, please contact Dojindo technical service.*

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