

# TransDiffer<sup>®</sup> Human Mesenchymal Stromal Cell Osteogenic Differentiation

Please read the datasheet carefully prior to use

Cat.No. MM301

Storage: at the proper storage temperature for one year

## Description

*TransDiffer*<sup>®</sup> Human Mesenchymal Stromal Cell Osteogenic Differentiation Medium is a serum-containing complete medium. It is suitable for differentiating Human Mesenchymal Stem Cells (MSCs) differentiate into osteoblasts. This product has strong universality and is suitable for MSCs from different sources (human pluripotent stem cells, umbilical cord, bone marrow, adipose) cultured in a variety of culture systems (serum-containing and serum-free). This product has good stability, the prepared complete medium can be stored stably for 1 month at 2-8°C. This product has high differentiation efficiency and can be used for the identification of osteogenic differentiation in the identification of multi-directional differentiation potential of mesenchymal stem cells.

## Kit Contents

Component	MM301-01	Storage
<i>TransDiffer</i> <sup>®</sup> Human Mesenchymal Stromal Cell Osteogenic Differentiation Basal Medium	180 ml	2-8°C
<i>TransDiffer</i> <sup>®</sup> Human Mesenchymal Stromal Cell Osteogenic Differentiation Supplement	20 ml	At -20°C in the dark, avoid repeated freeze-thawing

## Procedures

### Materials required but not included

Product Name	Catalog
PBS (1×)	TransGen, Cat. FG701-01
TrypLE <sup>™</sup> Express Enzyme (1×), no phenol red	Thermo Fisher, Cat. 12604054
<i>TransStem</i> <sup>®</sup> Serum-Free, Xeno-Free Human Mesenchymal Stromal Cell Medium	TransGen, Cat. MM101-01
Gelstain	Sigma, Cat. 1288485
<i>TransDetect</i> <sup>®</sup> Alizarin Red S Staining Kit (1%, pH4.2)	TransGen, Cat. MM302

### 1. Preparation of Osteogenic Differentiation of MSCs complete medium

Fully thaw 20 ml *TransDiffer*<sup>®</sup> Human Mesenchymal Stromal Cell Osteogenic Differentiation Supplement. After equilibrating to room temperature, add them to 180 ml *TransDiffer*<sup>®</sup> Human Mesenchymal Stromal Cell Osteogenic Differentiation Basal Medium. Mix well.

### 2. Preparation of 0.1% gelatin

Dissolve 5 g of gelatin in 500 ml of PBS, sterilize by autoclaving at 120°C for 20 minutes, and prepare 1% gelatin. After cooling, store at -20°C. Dilute with PBS according to the using amount of 0.1% gelatin when plating.

### 3. Petri dish coating

Add 0.1% gelatin to the culture dish to be coated, please refer to the table below for the coating amount, shake the liquid to cover the entire bottom. Then place in a 37°C incubator for at least 30 minutes. After 30 minutes, discard gelatin, and after the dish was air-dried, it was used to inoculate cells.

Notes: Gelatin-coated dishes can be stored at 4°C for two weeks under sterile and wet gelatin conditions.



Cell culture plate (dish)	Area	Coating amount per well
24-well	2 cm <sup>2</sup>	0.25 ml
12-well	4 cm <sup>2</sup>	0.5 ml
6-well	10 cm <sup>2</sup>	1 ml
35 mm	10 cm <sup>2</sup>	1 ml
60 mm	20 cm <sup>2</sup>	2 ml
100 mm	60 cm <sup>2</sup>	6 ml

#### 4. Differentiation process

(1) Digest well-grown MSCs into single cells with TrypLE™ Express Enzyme, seed the cells into pre-coated gelatin Petri dishes at a density of  $1 \times 10^4$  cell/cm<sup>2</sup>, and cultured with *TransStem*® Serum-Free, Xeno-Free Human Mesenchymal Stromal Cell Medium (Cat. No. MM101-01), and change the medium once a day.

(2) When the confluence of MSCs reached 100%, aspirate and discard the medium, and add osteogenic differentiation complete medium (1 ml/12-well plate) equilibrated to room temperature to initiate differentiation.

Notes: The initial differentiation time is generally 48-72 hours after inoculation. If the time is too long, it indicates that the cell proliferation ability is not good, and it is not suitable for osteogenic differentiation of cells.

(3) Change osteogenic differentiation complete medium every 5 days

Notes: In the later stage of differentiation, the cells are easily detached, and the medium should be changed gently.

(4) Observe the calcium nodule precipitation under the microscope, and make osteogenic differentiation identification.

Notes: For MSCs from different sources, the time course of osteogenic differentiation using *TransDiffer*® Human Mesenchymal Stromal Cell Osteogenic Differentiation Medium is different, as shown in the following table:

Source	Differentiation Time (days)
Human Pluripotent Stem Cells	21+
Bone Marrow	7-21
Adipose	7-21
Umbilical Cord	14-32

#### 5. Osteogenic differentiation identification

*TransDetect*® A lizarin Red S Staining Kit (1%, pH4.2) (TransGen, Cat. MM302) is recommended for osteogenic differentiation identification.

##### Notes

- The cell state of MSCs used for differentiation is an important factor affecting the efficiency of osteogenic differentiation. Please use MSCs with good growth conditions for osteogenic differentiation.
- *TransDiffer*® Human Mesenchymal Stromal Cell Osteogenic Differentiation Supplement cannot be subject to repeated freeze-thawing. Thoroughly thaw and equilibrate to room temperature before use. The prepared complete medium can be stored stably for 1 month at 2-8°C. Please divide it into a single-use amount and store it at -20°C before use. Avoid repeated freeze-thawing.

FOR RESEARCH USE ONLY

