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Technical Datasheet

EZStain™ Alkaline Phosphatase Staining Kit

Product Code: CCK077-1KT

1. Introduction

Mesenchymal stem cells are pluripotent stem cells and have the unique capacity to differentiate into different cell type- Osteocytes, Adipocytes and Chondrocytes. Osteoblast differentiation from mesenchymal precursors is characterized by the stages:

1) Cell proliferation
2) Matrix maturation
3) Matrix mineralization

Out of which matrix maturation is characterized by maximal expression of alkaline phosphatase. Hence, ALP can be used to monitor differentiation of MSCs into osteocytes.

2. About the Kit

The EZStainTM Alkaline Phosphatase Staining kit is designed to assess the differentiation of MSCs into osteoblasts qualitatively. This kit is based on the conversion of BCIP (5-Bromo-4-chloro 3-indolyl phosphate, disodium salt) and NBT (Nitroblue Tetrazolium Chloride) as substrate into violet colored insoluble NBT diformazan product by Alkaline Phosphatase which is produced as a result of differentiation of MSCs into osteoblasts.

3. Applications

- Identification of osteoblasts in tissue
- Identification of novel cellular factors / pathways involved in osteogenic differentiation
- Evaluation of effects of trophic factors, cytokines, and growth promoters, hormones, hormonal analogs and steroids on the differentiation process

4. Kit contents

Code	Contents	Quantity
CCK077(A)	Washing Solution	25ml
CCK077(B)	Washing buffer	25ml
CCK077(C)	Fixing Solution	25ml
CCK077(D)	Staining solution	339.5mg

Store all the reagents at room temperature

5. Materials required but not provided in the kit

- Mesenchymal stem cells
- Sterile water
- Microscope with 40X or higher objectives.
- Multi-well plates
- Serological pipettes

6. Directions for use

Users are advised to review entire procedure before starting the assay

Suggested working volumes of all reagents according to culture vessel

Culture vessel	Volume per well	No. of tests performed using 1 kit
96-well plate	75µl	260
48-well plate	150µl	130
24-well plate	300µl	60
12 well plate	500µl	40
6 well plate	1ml	20

6.1 General guidelines

- Do not leave the cell monolayer dry for more than 30 seconds during entire staining procedure.
- Gently add and remove all the reagents from the culture vessel to avoid detachment of cells from vessel surface. Add the reagents along the side of culture wells.
- Staining solution stains skin and clothing. Wear PPE (Personal Protective Equipment) while handling the solution.
- Controls:
  Include appropriate controls
  − Negative control: Undifferentiated cells
  - Positive control: Cells differentiated with known differentiation reagent

6.2 Procedure for staining cultured cells in plates

6.2.1 Washing the cells

1. Aspirate off the spent medium from control wells and osteogenic differentiated wells.
2. Add appropriate volume of washing solution (Part A) along the side of each well.
3. Swirl gently to wash the cell layer.

6.2.2 Fixation and permeabilization of cells

1. Aspirate off the washing solution and add appropriate volume of fixing solution (Part C) to each well.
2. Incubate the plate at room temperature for 60-90 seconds in fume hood.
3. After incubation aspirate off the fixing solution and add appropriate volume of washing buffer (Part B) along the side of each well.
4. Swirl gently to remove any traces of fixing solution.
5. Aspirate off the washing buffer and incubate the plate at room temperature for 2-3 minutes.

6.2.3 Staining the cells

Preparation of working stain solution (Part D)

1 Mix staining reagent (Part D) in 25ml of distilled water and vortex for few seconds.

2 Adjust the pH to 9.25-9.75.

3 Cover the substrate buffer with aluminum foil and store in dark.

a. Note: Working stain solution is stable for only 2 hours. Prepare only the required quantity just prior to use.

Procedure for staining

1. After incubation add appropriate volume of staining solution.
2. Incubate the plate at room temperature for 5-10 minutes.
3. After incubation aspirate off the staining solution and add appropriate volume of washing buffer (Part B) along the side of each well.
4. Swirl gently to remove the traces of staining solution.
5. Aspirate off the washing buffer and repeat washing with washing buffer till clear solution is obtained.
6. Add Wash solution (Part A) to each well and observe under phase contrast microscope at 40X magnification.

6.4 Procedure for staining tissue

1. Place a very thin piece of tissue on a clean, grease-free microscopy slide.
2. Mince it with the help of scalpel.
3. Very small unminced pieces of tissue can be left on the slide.
4. Place the slide on a sheet of tissue paper.
5. Put staining solution on the tissue in a quantity sufficient to cover the tissue.
6. Place long coverslip on the tissue across the slide. ***(Note**: Avoid trapping of bubbles while placing the coverslip in tissue. Presence of bubbles may interfere with microscopic observation.)*
7. Press the coverslip uniformly across the length of the slide to squash the tissue between coverslip and slide.
8. Incubate at room temperature for 30 minutes.
9. Fix the coverslip on slide with the help of nail polish.
10. Observe under phase contrast microscope at 40Xmagnification.

7. Interpretation of Observations

Figure 1
Undifferentiated Human Adult Mesenchymal Stem cells (40X)

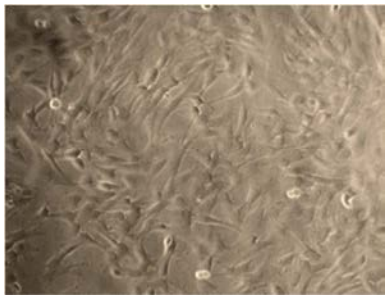
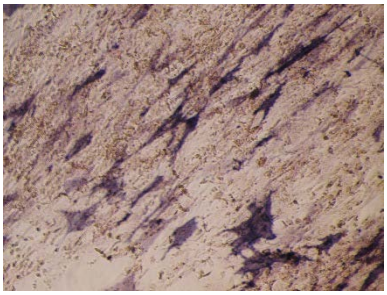


Figure 2
Differentiated Human Adult Mesenchymal Stem cells (40X)



8. Storage and Shelf Life

- Store all the reagents at -20ºC.
- If precipitation occurs in staining solution, filter it through Whatmann filter paper before use. Precipitation and subsequent filtration does not affect performance of the staining solution.
- Use before expiry date given on the label.

9. Related Products

HiOsteoXL™ Osteocyte Differentiation Medium (AL522)
HiFi™ Human Wharton's Jelly Mesenchymal Stem Cells (HWJ-MSC) (CL001)

10. Troubleshooting guide

Use the following troubleshooting guidelines for technical assistance

Problem	Cause	Solution
High background of staining in untreated cells	Inadequate washes after staining	Wash the cell layer with washing buffer until it is no longer purple/ violet in colour
	Precipitation in staining solution	Filter the staining solution through Whatmann filter paper before use
	Monolayer disturbed during addition or removal of media and reagents	Perform addition and removal gently along the side walls of the wells
Non-uniform staining	Cells growing in patches	Use uniformly spread confluent cells for staining