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Technical Datasheet

EZStain™ Osteocyte Staining Kit

Product Code: CCK030

Intended Use :

Identify osteocytes in tissue

1. Introduction

Osteogenesis is a process of development of new bone mass from undifferentiated tissues. During this process, calcium carbonate and calcium phosphates are deposited around osteoblasts, leading to formation of new bones. The process of osteogenesis is being extensively studied using different *in vitro* models to address the diseases such as *osteogenesis imperfecta* and osteoporosis. CCK030, EZstain™ Osteocyte Staining Kit is a ready-to-use kit developed for visualization of osteocytes or osteogenesis in cultured cells or tissue sections.

2. About the Kit

EZstainTM Osteocyte Staining Kit contains a complete ready to use collection of reagents required for osteocyte staining with Alizarin Red S. Alizarin Red S is an anthraquinone derivative which is used to identify calcium deposits in cells and tissue sections. Magnesium, manganese, barium, strontium and iron may interfere to a very small extent with staining. However, these elements usually do not occur in sufficient concentration to interfere with staining. Alizarin Red S represents a good marker to confirm calcium deposition and bone matrix formation.

3. Applications

EZStain™ Osteocyte staining kit can be used to identify osteocytes in tissue,study intracellular signalling pathways regulating osteogenic differentiation and effect of toxic agents growth factors and drugs on osteogenic process.

4. Kit contents

Contents	Quantity
Washing Solution	1 X 20ml
Fixing Solution	1 X 20ml
Staining solution	1 X 20ml

Store all the reagents at room temperature

5. Materials required but not provided in the kit

- Osteocytes / Appropriate tissue
- Sterile water
- Microscope with 40X or higher objectives
- Microscopy slides
- Cover slips
- Scalpel
- 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.
- Add the reagents gently along the side of culture wells.
- Wear PPE (Personal Protective Equipment) while handling the staining solution.
- Controls:
  Include appropriate controls-
  -Negative control: Undifferentiated cells
  -Positive control: Cells differentiated with known differentiation reagent

Note: pH of Alizarin Red S is very critical for optimum staining of calcium deposits. Before use, ensure that the pH of staining solution is between 4.1 to 4.3. If pH is found to be deviated from the specified range, adjust it to 4.1 to 4.3 before use.

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 along the side of each well.
3. Swirl gently to wash the cell layer.

6.2.2 Fixation of cells

1. Aspirate off the washing solution and add appropriate volume of fixing solution to each well.
2. Incubate the plate at room temperature for 30 minutes in fume hood.
3. After incubation, aspirate off the fixing solution and add appropriate volume of distilled water along the side of each well.
4. Swirl gently to remove any traces of fixing solution.

6.2.3 Staining the cells

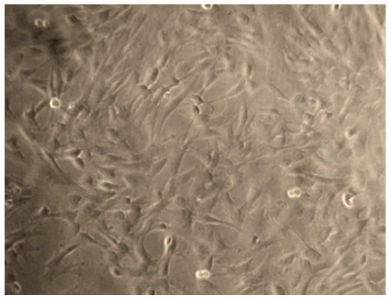
1. Aspirate off the water and add appropriate volume of staining solution and incubate at room temperature for 45 minutes in dark.
2. After incubation aspirate off the staining solution and add appropriate volume of distilled water along the side of each well.
3. Swirl gently to remove any traces of staining solution.
4. Aspirate off the water and repeat washing with water till clear solution is obtained.
5. Again add distilled water to each well and observe under phase contrast microscope at 40X magnification.

6.3 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 transparent nail polish.
10. Observe under phase contrast microscope at 40X magnification.

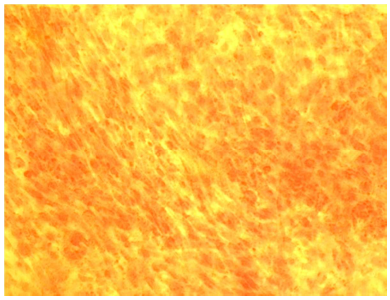
7. Observations

Figure 1



Undifferentiated Human Adult Mesenchymal Stem cells (40X)

Figure 2



Calcium deposits in mesenchymal stem cells differentiated into osteocytes stained red in colour by Alizarin Red S (40X)

8. Storage and Shelf Life

- Store all the reagents at room temperature.
- 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

HiDiff™ 3T3-L1 Differentiation kit
Code: CCK011-100NO

Xpert™ 3T3-L1 Differentiation Teaching Kit
Code: CCK021-25NO

EZstain™ Adipocyte Staining Kit
Code: CCK013-1KT

EZstain™ Chondrocyte Staining Kit
Code: CCK029-1KT

EZstain™ Hepatocyte Staining Kit
Code: CCK014-1KT

EZstain™ Chondrocyte Staining Kit
Code: CCK029-1KT

10. Troubleshooting guide

Use the following troubleshooting guidelines for technical assistance

Problem	Cause	Solution
High background of staining in untreated cells (negative control cells i.e. non-osteogenic cells)	Inadequate washes after staining	Wash the cell layer with distilled water until it is no longer red/ pink in colour
	Precipitation in staining solution	Filter the staining solution through Whatmann filter paper before use
Non-uniform staining	Monolayer disturbed during addition or removal of media and reagents	Perform addition and removal gently along the side walls of the wells
	Cells growing in patches	Use uniformly spread confluent cells for staining

In vitro diagnostic medical device

CE Marking

Consult instructions for use

Do not use if package is damaged

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Single use.
Not intended to be reprocessed and/or used on another patient

Sterilized using aseptic processing techniques

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