COSMO BIO CO., LTD.
Inspiration for Life Science

For research use only, Not for diagnostic use.

Catalog No. CSR-ARD-SET

Calcification Evaluation Set

This product utilizes Alizarin Red dye solution to stain calcified nodules produced by differentiated osteoblasts, and an acid solution to extract dye bound to calcified nodules. The optical absorbance of the extracted dye solution is then measured, providing a convenient assay to evaluate the extent of osteoblast differentiation (calcification) in cultured cells.

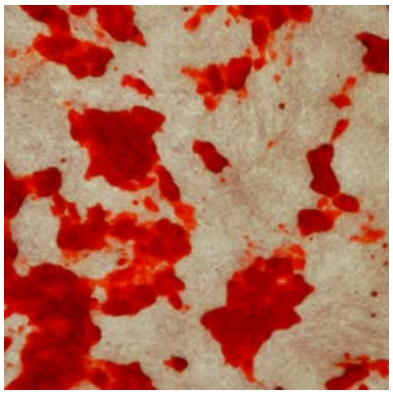
1) Component

①Alizarin Red Solution (Product Code：**CSR-ARD-A1,** 100mL, 1 bottle, store at room temperature)
②Calcified Nodule Extraction Solution (5% Formic Acid, Product Code：**CSR-ARD-E1**, 100mL, 1 bottle, store at room temperature)

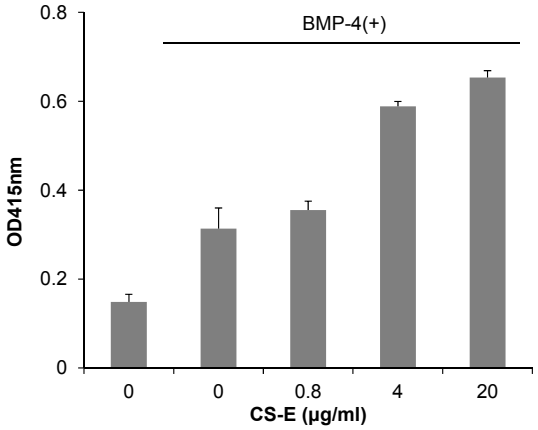
2) Assay Procedure

(1) Remove culture medium from each well (24-well or 48-well plate), then once with PBS.
(2) Fix washed cells with 10% neutral buffered formalin or 4% paraformaldehyed for app. 10 minutes.
(3) Remove fixative solution and wash wells with water.
(4) Remove remaining water carefully by aspiration or by patting the plate on a paper towel.
(5) Add Alizarin Red solution to each well (0.5mL/well for 24-well plate or 0.25mL/well for 48well plate), and stain for 30 minutes at room temperature.
(6) Remove the Alizarin Red solution in each well and wash with water until wash water is nearly clear (pale red color. Wash water will not clear completely).
(7) Carefully remove all remaining water from wells by aspiration or by patting the plate on a paper towel.
(8) Add Calcified Nodule Extraction Solution to each well (0.5mL/well for 24-well plate or 0.25mL/well for 48-well plate), and shake plate gently for about 10 minutes on a plate shaker to extract the bound dye.
(9) Transfer 100μL of the extracted dye solution to 96-well plate and measure the absorbance at 400～450nm on a plate reader.

3) Application Example



Alizarin Red staining of MC3T3-E1 cells cultured in differentiation medium



The Effects of BMP and chondroitin sulfate E (CS-E) on the mineralization of MC3T3-E1 cells (Mean±SD)

Cautions

(1) When using this reagent set, take appropriate safety precautions (i.e., lab coat, gloves, eye goggles, etc) to avoid contact with body.
(2) If upper internal surface of culture wells are strongly stained by Alizarin Red due to non-specific calcium adherence, remove this red material using a cotton swab or other wipe prior to Step (8).
(3) This reagent set is not suitable for use with calcium phosphate coated plates such as CSR-BRA-24P or CSR-BRA-48P.

www.cosmobio.co.jp

5) Reference

(1) Miyazaki T. et. al. Oversulfated chondroitin sulfate-E binds to BMP-4 and enhances osteoblast differentiation. J Cell Physiol. 217: 768-777. 2008.

Products:

Product code	Product name	Quantity	Remarks
CSR-ARD-SET	Calcification Evaluation Set	1 Set	store at room temperature
CSR-ARD-A1	Alizarin Red Solution	100mL	store at room temperature
CSR-ARD-E1	Calcified Nodule Extraction Solution	100mL	store at room temperature

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Manufactured by PG Research

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