



**Alizarin Red S Stain (2%)
(ARed)**
Catalog #0223

Product Description

Alizarin Red S is an anthraquinone dye used to stain for calcium deposits, which are indicators of mature osteocytes. The dye forms a complex with calcium during the process of chelation resulting in birefringence [1]. 2% Alizarin Red S Stain (ARed) is a convenient, ready-to-use solution.

Materials Supplied by User (Not provided)

- 4% Paraformaldehyde solution in Phosphate Buffered Saline (PBS)
- Deionized H₂O (diH₂O)

Product use

ARed is for research use only. It is not approved for human or animal use, or for application in *in vitro* diagnostic procedures.

Storage

Room temperature.

Shipping

Room temperature.

References

[1] Clark, G. (Ed.). (1981). *Staining Procedures*. (4th ed.). Baltimore, MD: Williams & Wilkins.

Procedure

A. Preparation of Cells:

1. Aspirate culture medium from each well gently without disrupting the cells.
2. Wash the cells twice with 1 mL PBS and gently aspirate.
3. Fix the cells in 4% Paraformaldehyde in PBS for 15 min at room temperature.
4. Carefully remove the fixative and wash the cells 3 times with diH₂O.

B. Staining of Cells:

1. Remove diH₂O completely and slowly add 1 mL of 2% Alizarin Red S Stain Solution to each well.
2. Incubate for 20-30 minutes at room temperature.
3. Remove dye and wash 3-5 times with diH₂O.
4. Add 1 mL diH₂O to each well to keep cells from drying out. Samples are now ready for imaging under microscope.

(a)

(b)

Figure 1. (a) Human mesenchymal stem cells from bone marrow (HMSC-bm, Catalog # 7500) were cultured in growth medium, complete Mesenchymal Stem Cell Medium (MSCM, Catalog # 7501) for 21 days. Alizarin Red staining was not detected (Magnification: 100X). (b) HMSC-bm were cultured in complete MSC Osteogenic Differentiation Medium (MODM, Catalog # 7531) for 21 days. The Alizarin Red staining demonstrated the presence of calcium deposits in cells (Magnification: 100X).