Alizarin Red S Staining Quantification Assay (ARed-Q)

Catalog #8678
100 Tests

Product Description

Alizarin Red S (ARS), an anthraquinone dye, has been widely used to evaluate calcium deposits in cell culture. The ARS staining is quite versatile because the dye can be extracted from the stained monolayer of cells and readily assayed.

ScienCell's ARS Staining Quantification Assay (ARed-Q) provides a sensitive tool for the recovery and semi-quantification of ARS in a stained monolayer of cells. Mineralization is assessed by extraction of calcified mineral at low pH, neutralization with ammonium hydroxide, and colorimetric detection at 405 nm in a 96-well format. This assay is more sensitive than the cetylpyridinium chloride (CPC) extraction method, improving the detection of weakly mineralizing monolayers [1]. It provides a wider linear range: destained ARS dye ranging from 30 µM to 4 mM shows a linear relationship with the absorbance at 405 nm, making dilutions of samples prior to measurement unnecessary.

We have applied this quantification assay to the osteogenesis induction of human mesenchymal stem cells (hMSCs). Cells were cultured in different osteogenic differentiation medium for 18 days, fixed for ARS staining and quantified for mineral deposit using the kit. There is significant difference between the untreated control, the induced samples, and the different induction conditions (Figure 2). ScienCell's ARed-Q assay can be applied for mesenchymal stem cell osteogenic differentiation, tumor characterization, and osteognic compound screening.

Kit Components

Cat. No.	Reagent	Quantity	Storage
8678a	40 mM Alizarin Red S	100 mL	Room Temperature
8678b	10% Acetic acid	80 mL	Room Temperature
8678c	10% Ammonium hydroxide	20 mL	Room Temperature
8678d	Standard dilution solution	100 mL	Room Temperature

Materials Not Included in Kit

Phosphate buffered saline (PBS)
4% Paraformaldehyde solution
Deionized H_2O (diH_2O)
Mineral oil (optional)

Quality Control

A serial dilution of ARS with concentration ranging from 31.3 µM to 2 mM shows a linear relationship with the absorbance at 405 nm (Figure 1).

Product Use

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

Shipping

All components are shipped at room temperature.

References

[1] Gregory CA, Gunn WG, Peister A, Prockop DJ. (2004) "An Alizarin red-based assay of mineralization by adherent cells in culture: comparison with cetylpyridinium chloride extraction." *Analytical Biochem*. 329: 77-84.

Procedures

Caution: The reagents containing either acetic acid or ammonium hydroxide have strong odors and are irritating to eyes and skin. We recommend wearing proper personal protective equipment including lab coats, masks, gloves and safety glasses when handling these chemicals.

Note: The following procedures are optimized for 6-well plates. Indicated volumes are for one well of a 6-well plate, volumes in ( ) are for one well of a 24-well plate. For all other applications, please adjust volumes accordingly.

A. Alizarin Red S staining

1. Remove culture medium from each well and gently wash cells 3 times with 1xPBS.
2. Fix the cells in 4% formaldehyde for 15 minutes at room temperature.
3. Remove fixative and wash the cells 3 times with diH_2O.
4. Remove diH_2O completely and add 1 mL of 40 mM ARS per well. Incubate at room temperature for 20 - 30 min with gentle shaking.
5. Remove the dye and wash the cells 5 times with diH_2O.
6. If required, inspect the cells using a phase microscope and take images. (Optional)
7. Tilt the plates for 2 min to facilitate removal of excess water.
8. Store plates at -20°C prior to dye extraction.

B. Preparation of samples

1. Add 800 µL of 10% acetic acid to each well of a 6-well plate and incubate at room temperature for 30 minutes with shaking (200 µL per well for a 24-well plate).
2. Collect the cells using a cell scraper and transfer the cells in 10% acetic acid to a 1.5-mL microcentrifuge tube.
2. Vortex for 30 seconds.
3. Heat samples at exactly 85°C for 10 minutes. To avoid evaporation, the tubes may be sealed with parafilm or add 500 µL of mineral oil to the top of the slurry.
4. Incubate tubes on ice for 5 minutes.
   Caution: Do NOT open the tubes until fully cooled.
5. Centrifuge the slurry at 20,000g for 15 minutes.
6. While centrifuging, prepare ARS standards as in Part C. (Optional)

7. After centrifugation, transfer 500 µL (or 200 µL) of the supernatant to a new tube.

8. Add 200 µL (or 75 µL) of 10% ammonium hydroxide to neutralize the acid.
   NOTE: pH should be between 4.1 and 4.5. Take a small aliquot and test to ensure pH falls within the range.

9. Aliquot 150 µL per well (or 50 µL/well) of the samples and standard prepared in Part C in triplicates in a 96-well plate (opaque-walled, transparent-bottomed plates). Read the absorbance at 405 nm with a plate reader.

10. Plot an ARS standard curve using Excel and calculate the ARS concentration of the samples. (Optional)

C. Preparation of Alizarin Red S standard (optional)

Note: If the exact ARS concentration is not needed, the measurement of the ARS standard curve can be skipped.

1. Add 100 µl of 40 mM Alizarin Red S solution to 900 µl of standard dilution solution to make a 1 mL solution of 4 mM ARS. Mix well.

2. Obtain 8 1.5-mL microcentrifuge tubes, add 500 µl of standard dilution solution into each tube and label them #1 through #8.

3. Add 500 µl of the 4 mM ARS solution into tube #1 and mix well to get the 2 mM ARS standard.

4. Transfer 500 µl of the 2 mM ARS standard from tube #1 to tube #2 and mix well to get the 1 mM ARS standard.

5. Repeat step 4 for tubes #3-7 to serially dilute the ARS standards. Do not add any ARS to tube #8, which serves as the blank.

6. Obtain a 96-well plate, prepare 3 replicates (A, B, C) of each ARS standard by aliquoting 150 µl/well of each ARS standard into triplicate wells of the 96-well plate, according to the following plate format:

	#1	**#2**	**#3**	**#4**	**#5**	**#6**	**#7**	**#8**
A	2 mM	1 mM	0.5 mM	0.25 mM	0.125mM	0.0625 mM	0.0313 mM	Blank
B	2 mM	1 mM	0.5 mM	0.25 mM	0.125mM	0.0625 mM	0.0313 mM	Blank
C	2 mM	1 mM	0.5 mM	0.25 mM	0.125mM	0.0625 mM	0.0313 mM	Blank

D. Calculations (Optional)

1. Average the OD_{405nm} of replicate wells of each ARS standard, blank and samples. Subtract the average OD_{405nm} value of the blank from the average OD_{405nm} values of others.

2. Based on the calibrated OD_{405nm} value of the ARS standard, make a standard curve by plotting absorbance (405 nm) as a function of ARS concentration. (See Figure 1 for a typical standard curve.) Determine the equation and R^2 value of the trend line.

3. Calculate the ARS concentration in the samples according to the equation of the trend line.

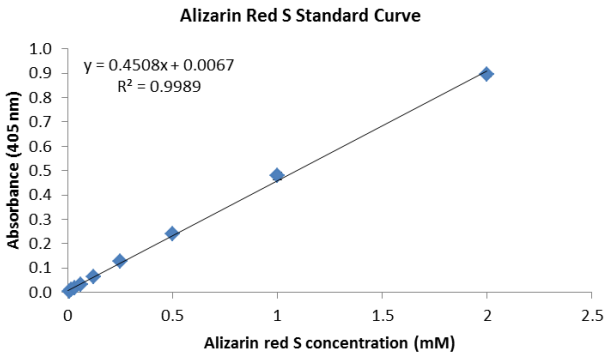

Figure 1. A typical Alizarin Red S standard curve measured by ScienCell's Alizarin Red S Staining Quantification Assay.

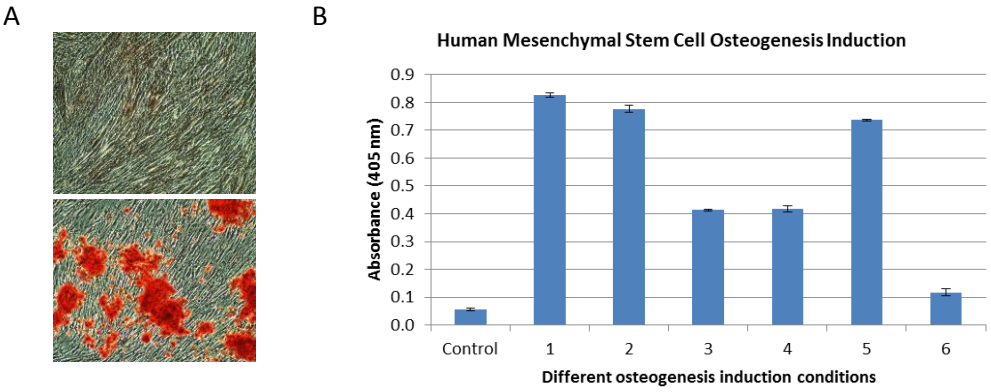

Figure 2. ARS staining and quantification of human mesenchymal stem cell (hMSC) osteogenesis. Human MSCs were treated under 6 different conditions (sample 1 – 6) to induce osteogenesis for 18 days. ScienCell's Alizarin Red S staining quantification assay was applied to the treated samples and controls cultured in the hMSC growth medium without induction. A. Typical ARS staining for controls (top) and treated samples (bottom); B. ARS quantification using the assay. Data are expressed as the mean (n = 6) with error bars representing standard deviations.