

## Bone Cells Core

Director: Paola Divieti Pajevic, MD, PhD

Email: [bonecellcore@partners.org](mailto:bonecellcore@partners.org)

# Alizarin Red & Quant Assay

### Alizarin Red

1. Rinse 2X with PBS,
2. Fix the cells in ice-cold 70% ETOH for 1 hr
3. Rinse 2X with H<sub>2</sub>O
4. Stain with Alizarin red stock solution for 10 min at RT with gentle rotation
5. Rinse 5X with H<sub>2</sub>O
6. Rinse 1X with PBS for 15 min to remove non specific staining

AR- stock= 1% alizarin red in H<sub>2</sub>O pH 4.2 (important!) Better to filter with a paper filter before first use

### protocol:

Destain cells with 10% CPC for 15 min with rotation @ RT  
transfer 200ul of the diluted sample into 96 well  
read at 562

### Standard curve (200ul/well)

- |             |                                      |
|-------------|--------------------------------------|
| 1. 0.46 mM  | 24ul stock solution +2976 of 10% CPC |
| 2. 0.34 mM  | 1500 ul from 1 + 500 ul of 10% CPC   |
| 3. 0.17 mM  | 500 ul from 2 + 500 ul of 10% CPC    |
| 4. 0.11 mM  | 667 ul from 3 + 333 ul of 10% CPC    |
| 5. 0.027 mM | 350 ul from 4 + 1050 ul of 10% CPC   |
| 6. 0.013 mM | 550 ul from 5 + 550 ul of 10% CPC    |
| 7. 0.007 mM | 400 ul from 6+ 400 ul of 10% CPC     |

10% CPC = 1 g Cetylpyridinium chloride CPC in 10 ml of Sodium Phosphate 10mM pH 7