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Musculoskeletal Cell Core

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Alizarin Red and 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 H₂O
4. Stain with Alizarin red stock solution for 10 min at RT with gentle rotation
5. Rinse 5X with H₂O
6. Rinse 1X with PBS for 15 min to remove non specific staining

AR- stock= 1% alizarin red in H₂O 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