PROTOCOL

How to verify osteogenesis on P3D Scaffolds

— Studying osteoblast differentiation using an ALP staining assay

Application

Mesenchymal stem cells express alkaline phosphatase (ALP) which increases during osteoblast differentiation, and ALP levels can therefore be used as osteogenic marker. For more protocols, please visit our Resources Platform.

General advice on material handling

The fixing solution contains formaldehyde, please perform this procedure inside a fume hood.

Materials

- Trypsin
- PBS
- PBST
- Trypan blue
- Distilled water
- ALP staining kit (ab242286 Alkaline Phosphatase Staining Kit (Red) from Abcam.)
- Microscope

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Procedure

1. Prepare reagents

    a. Alliquot 30 mL Fixing Solution and 15 mL Staining Solution A and B. Place alliquots at RT for 30 min. Spin down.

    b. Prepare 240 mL 1x PBST by adding 120 uL Tween-20 to 240 mL PBS.

    c. Make 1x working solution by mixing Staining Solution A and B 1:1. Make the volume required for your setup (0.3 mL - 0.8 mL pr sample depending on well size, scaffolds must be completely covered)

2. Aspirate medium from cells and wash twice in 2 mL 1. PBST. Aspirate.

3. Add 0.3-0.8 mL Fixing Solution and incubate at RT for 2 min

4. Aspirate Fixing Solution and wash twice in 2 ml 1x PBST. Aspirate.

5. Add 0.3-0.8 mL freshly prepared Staining Solution. Incubate at RT for 15-30 min protected from light.

6. Remove the Staining Solution and wash twice in 2 mL 1x PBS

7. Evaluate presence of osteoblast with light microscope. Red indicates ALP presence and may be quite strongly labelled.

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