UNIVERSITÄT BERN

CALCEIN STAINING

Protocol used for calcein bulk staining of microcracks

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Protocol purpose:
This protocol is aimed to stain in vivo microcracks by performing bulk staining of 3 mm thick bone samples using calcein. Calcein binds to free calcium present at the surface of microcracks and bulk staining allow to stain microcracks present in vivo only, not the ones generated by sample preparation.

PROTOCOL

Step 1 [~30 + 5 minutes minutes]

Cutting

- Cut bone sample using a diamond band saw into slices of 3 mm thickness.

Step 2 [~30 + 3 days minutes]

Fixation

- Put the samples in 4% formaldehyde for two days and rinsed for 8-12 hours in tap water. Exchanged water four times in the rinsing step.

Step 3 [~? + ? minutes]

Bulk staining

- Dissolve calcein at a concentration of 0.5 mM. Dissolution is performed either in distilled water or in 100% ethanol.
- Perform bulk staining under low vacuum (-300 mbar) at room temperature. 2 samples are stained for 7 hour and 2 are for 24 hours.

Step 4 [~? + ? minutes]

Embedding

- Do something

Step 5 [~? + ? minutes]

Sample preparation

- Do something

Step 6 [~? + ? minutes]

Histology analysis

- Do something

WARNINGS

Calcein
The $C_{30}H_{26}N_2O_{13}$ is bad for you. In case of contact, wash directly and inform the safety responsible

Human tissue
Human bone can transmit diseases. Always use gloves when manipulating

NOTES

REFERENCES