OsteoSite

Dentine Discs

Resorbable Dentine Substrate

For Research Use Only

Code AE-80100 100 disc pack
AE-8050 50 disc pack

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PTHrP Far C-Terminal, PTHrP (144-152)
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Dentine Discs (Resorbable Dentine Substrate)

Product

Discs of devitalised dentine for use as bone resorption substrates. The discs are supplied as 5 mm diameter wafers of dentine (nominal thickness 0.3 mm) Each batch of substrates is tested to ensure resorbability by functional osteoclasts. A pre-resorbed example disc is available on request in order to facilitate identification of *in vitro* resorption.

Presentation

Discs are supplied in packs of 50 in sterile screw-capped containers. The discs are sterilised by ultraviolet irradiation and should only be opened in a sterile environment such as a laminar flow cabinet.

Storage

This product can be stored at room temperature. If the containers are not opened and the discs are kept dry, there is no perceptible deterioration for the stated shelf-life.

Application

This product has been designed to permit the *in vitro* quantification of bone resorption using the "bone slice assay" (1,2) in which cultured osteoclasts excavate authentic resorption lacunae on the mineralised substrate. In the past 16 years since the first description of this assay (1,2), it has been widely used to investigate the effects of many factors on bone resorption and is now the most widely used method of investigating bone resorption *in vitro* (3-8).

The bone slice assay can be used with osteoclasts isolated from bones of experimental animals including mice, rats, chicks, rabbits and cats. It has been widely used to investigate resorption by human osteoclasts either isolated directly from bone or grown from marrow cultures (4).

One of the major recent advances in the field has been the development of a technique to induce osteoclast formation from peripheral blood cells by the addition of RANK ligand (9). Using this technique large numbers of functional osteoclasts can be generated which will excavate resorption lacunae in mineralised substrates including OsteoSite resorbable dentine discs. Resorption can easily be quantified by reflected light microscopy (3,4,6,8).

References

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3. Walsh C A, Beresford J N, Birch M A, Boothroyd B and Gallagher J A (1991) Application of reflected light microscopy to identify and quantitate resorption by isolated osteoclasts. J. Bone Miner. Res. 6:661-671.
4. Walsh C A, Carron J A and Gallagher J A (1996) The isolation of osteoclasts from human giant cell tumours and long-term marrow cultures. In 'Human Cell Culture Protocols' Ed. G.E. Jones, Humana Press Inc. pp 233-262.
5. Arnett T R and Dempster D W (1987) A comparative study of disaggregated chick and rat osteoclasts in vitro: Effects of calcitonin and prostaglandins. Endocrinology 120:602-608.
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8. Tamura T, Takahashi N, Akatsu T, Sasaki T, Udagawa N, Tanaka S and Suda T (1993) New resorption assay with mouse osteoclast-like multinucleated cells formed in vitro. J. Bone Miner. Res. 8:953-960.
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Generic Instructions For Use

1. Dentine discs are supplied in sterile plastic containers. These should only be opened in a sterile environment, such as a tissue culture cabinet.

2. After opening the container, the sterile plug of cotton wool should be removed using a pair of sterile fine forceps. The discs should then be transferred to a sterile petri dish by inverting the tube.

3. Transfer the discs to the culture dishes in which they will be used, using fine forceps and taking care not to scratch the surface of the dentine. The discs have been designed for use in standard 96 well tissue culture plates, but they can be used in any type of tissue culture container.

4. Before addition of cells, the dentine discs should be pre-wetted for at least one hour. Sterile phosphate buffered saline or tissue culture medium should be used.

5. The pre-wetting solution should be removed and the cell suspension added. The number of cells added depends on the source of the osteoclasts and the experimental design. Resorption is analysed by light microscopy and it is possible to measure the activity of a single osteoclast. However, in most experimental protocols it will be appropriate to add more osteoclasts (but generally less than 200 per disc).

6. The settling times used will also depend on the source of osteoclasts and the experimental design. Mature osteoclasts will attach to the dentine discs within one hour, but longer settling times are used in many protocols. Medium can be changed by using standard tissue culture techniques and equipment including automatic pipettes.

7. Resorption by mature osteoclasts will be detectable after 12 hours of culture. The period of culture will depend on the experimental design.

8. If aqueous fixatives are used, the dentine discs can be fixed in the tissue culture containers. Withdraw the conditioned medium and wash the tissue culture well two times with phosphate buffered saline or tissue culture medium and then add the fixative. A widely used fixative is 4% glutaraldehyde in 0.2M sodium cacodylate solution.

9. Resorption pits can be identified following staining with a variety of standard histological stains. We recommend 1% toluidine blue in 0.5% sodium tetraborate solution. Dentine discs should be stained for 3 minutes in this toluidine blue solution. Excess staining can be removed by washing, followed by rinsing in tap water and then airdrying. Stained discs can then be stored indefinitely at room temperature.

10. Resorption lacunae can be identified in a number of ways, including scanning electromicroscopy and light microscopy. We recommend the use of reflected light microscopy.

11. Users should consult some of the recommended texts to determine which techniques are most suitable for the application they require.

Example Protocol For Generation Of Human Osteoclasts *In Vitro*

This method uses 20 mL of peripheral blood, which gives sufficient osteoclasts for approximately 50 dentine discs.

1. Prepare the sterile dentine discs and place in the wells of a 96 well plate, 1 disc per well. Add 100 µL of αMinimal Essential Medium (αMEM) plus 10% Fetal Calf Serum (FCS) to each well to pre-wet the discs.

2. Mix 20 mL of heparinised peripheral blood with 20 mL of αMEM. Prepare 5 Falcon tubes with 5 ml of Histopaque (Sigma) and add 8 ml of the peripheral blood cell suspension to each tube. Spin at 450 g for 30 minutes and decant the buffy coat layer.

3. Wash the buffy coat layer in 10 mL of αMEM and spin at 1500 rpm for 20 minutes. Re-suspend the cell pellet in 4 mL of αMEM plus 10% FCS and count the cells using a haemocytometer (NB: cell concentration will depend on the efficiency of buffy coat removal).

4. Once the cells have been counted add 500,000 cells in approximately 20 µl of medium to each well of the 96 well plate. Place in the incubator and leave for one hour.

5. At the end of the incubation period wash each well 3 times by withdrawing the medium and adding fresh medium. Finally replace the medium with 100 µl of new medium made up of αMEM plus 10% FCS with the addition of RANK ligand (30 ng/mL) and M-CSF (25 ng/mL).

6. Change the medium every 2-3 days. Osteoclasts will develop over a 2 week period and this process can be monitored by removing discs and staining them.

7. During the first 2 weeks of culture the effects of potential activators or inhibitors of osteoclastogenesis can be added to the medium.

8. In the third week of culture there is extensive resorption of the dentine discs. The effects of potential inhibitors or activators of bone resorption can be assessed during this period.

9. Discs are stained with toluidine blue and the extent of resorption is assessed by reflected light microscopy.