ABGENEX Pvt. Ltd.,
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17-1001: Celltase™ Cell Detachment Solution

Description

Celltase™ is a natural enzyme mixture with proteolytic and collagenolytic enzyme activity. This means it mimics the action of trypsin and collagenase at the same time. However, because it is more efficient than mammalian trypsin & collagenase, it is formulated at a much lower concentration making it less toxic and gentler, but just as effective.

Advantages of Accutase

- Can be used whenever gentle and efficient detachment of any adherent cell line is needed. Accutase is a direct replacement for trypsin.
- Works extremely well on embryonic and neuronal stem cells; mono layers of stem cells can be grown after passaging with Accutase.
- Preserves most epitopes for subsequent flow cytometry analysis.
- Does not need to be neutralized when passaging adherent cells. The addition of more media after the cells are split dilutes Accutase so it is no longer able to detach cells.
- Does not need to be aliquoted. A bottle is stable in the refrigerator for 2 months.

Cell Lines tested

A few cell lines that Accutase has been shown to detach without harm: hESCs, fibroblasts, keratinocytes, vascular endothelial cells, hepatocytes, vascular smooth muscle cells, hepatocyte progenitors, primary chick embryo neuronal cells, bone marrow stem cells, adherent CHO and BHK cells, macrophages, 293 cells, L929 cells, immortalized mouse testicular germ cells, 3T3, Vero, COS, HeLa, NT2, MG63, M24 and A375 metastatic melanoma, gliomas U251 and D54, HT1080 fibrosarcoma cells, and Sf9 insect cells.

Product Info

Amount :	500 / 100 ml
Content :	1X Celltase enzyme solution containing aqueous buffer, EDTA and Phenol Red. Sterile-filtered, suitable for cell culture.
Storage condition :	2 months at 2-8°C. 12 month if frozen at -20°C. For long term or intermittent use the product may be aliquoted and frozen if necessary.

Application Note

Recommended procedure for general dissociation:

Proper aseptic techniques should be followed while handling cell lines and reagents including Celltase.

1. Thaw Celltase at room temperature or at 4°C overnight. Gently swirl the Celltase bottle for proper mixing.

2. Aspirate media and wash the cell monolayer with 4 mL of sterile DPBS (w/o calcium and magnesium). Aspirate DPBS from the tissue culture flask.

3. Add Celltase to flask (10 mL per 75 cm^2

surface area) using aseptic procedures.

4. Incubate the culture flask at room temperature for 5 to 10 minutes up to a maximum of 1 hr. The cells incubated with Celltase can be left on ice for several hours.

5. Inspect under the microscope for signs of cell detachment like shrinkage or rounding.

6. Detach the cells either by pipetting up and down several times or smack the flask against palm of hand once or twice.

7. (Optional) 20 µl sample of the cell suspension can be used to determine the viable cell density.

8. Resuspend the cells in fresh media and split into new flasks as is needed. Incubate at 37°C in a humidified CO_2 incubator. (Celltase is auto-inhibitory at 37°C. Hence quenching with media as done in case of trypsin is not needed for routine passages)

9. The cells will re attach at 37°C within a few minutes depending upon cell type.

Dissociation of human ESCs grown in Serum Free Media on hESC-qualified Basement Membrane Extract.

1. Aspirate the media from culture dish and wash with 4 mL of sterile DPBS (w/o calcium and magnesium).

2. Aspirate DPBS and add 2 mL of Celltas to culture dish.

3. Incubate for 2 to 5 minutes at room temperature until individual single cells start to round up. Inspect under the microscope for signs of cell detachment. Incubate longer if you observe incomplete detachment.

4. Gently rinse to remove cells from the surface of the plate. Transfer cell suspension to 15 mL conical tube. Pipette up and down gently until cells are in a single cell suspension.

5. Rinse surface of the dish for any remaining cells using media and transfer to the conical tube from Step 5.

6. (Optional) Take a 20 µl sample of the cell suspension to determine viable cell density.

7. Centrifuge conical tube containing the cell suspension at 1000 RPM for 4 minutes in a swing bucket rotor.

8. Aspirate supernatant and resuspend the cells in fresh medium and plate on coated dish. Incubate at 37°C in a humidified CO_2 incubator.

Dissociation of adherent human or rat neuronal stem cells grown in Serum Free Media on coated dishes is also performed in a similar manner.

Note: Among different cell lines adherence to tissue culture plastic might vary. Hence the incubation time required for dissociation should be determined for specific cell type and application

Precautions:

Celltase is temperature sensitive. Do not store Celltase™ at room temperature. Upon receipt the product should be kept at -20°C for long term storage and at 4°C for short term storage. It is recommended to thaw Celltase™at 4°C overnight or in a bath of cool water but never at 37°C.

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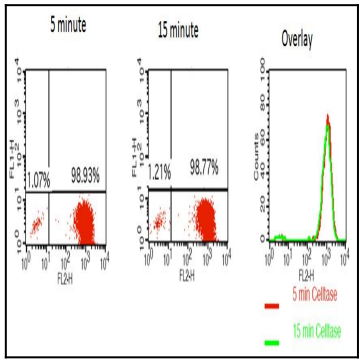

Fig. 1: Human A431 epidermoid carcinoma cells cultured in DMEM + 10% FBS were treated with Celltase at room temperature for different time points. Treatment resulted in rapid cell detachment and high viability. Cell viability was 96±4% even after 60 minutes of Celltase treatment.

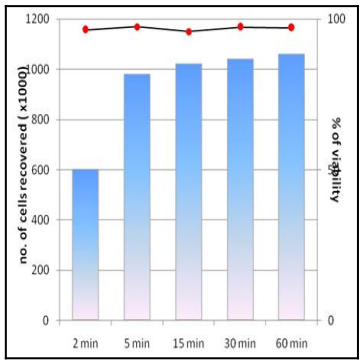

Fig. 2: A431 cells were detached with Celltase @ RT with 5 and 15 minutes of incubation time. Cells were washed, harvested and stained using anti-Epcam antibody (Catalogue no: 10-7515). Anti mouse PE was used as secondary antibody. Cells were analyzed by flow cytometry and both the files were overlapped. Expression of Epcam antigen on the cell surface after Celltase treatment was similar in both the time points. Celltase is gentle on surface antigens and cell membrane unlike Trypsin.