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National Institute for Cellular Biotechnology

BriClone

Hybridoma Cloning Additive

Protocol for Use

Introduction

BriClone is a sterile filtered media supplement for use in the post-fusion stages of hybridoma cloning.

Shelf Life and Storage

BriClone is stable at -20˚C until the expiry date (see on the label).

It is recommended to aliquot this product into single use volumes to avoid repeated freeze-thaw cycles.

Once thawed, BriClone is stable for 1 month at +4˚C.

Thawing

Place your bottle or aliquot of BriClone in a waterbath at 37˚C until fully thawed or overnight in the refrigerator.

Hybridoma Growth Post-Fusion

1. Perform the fusion of mouse splenocytes and myeloma cells (i.e Sp2 cells) according to your laboratory procedure.
2. Centrifuge the cells at 500 rpm for 5 minutes to remove polyethylene glycol.
3. Resuspend the freshly fused hybridomas in the selective medium supplemented with 5% BriClone.
4. Plate the cells in a 48 well plate in 800µl.
5. Incubate for 12 days undisturbed at 37˚C.
6. After 12 days of growth, check the presence of colonies under the microscope. You can also check the production of the antibody.

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Hybridoma Cloning

The hybridomas can be cloned under limiting dilution.

1. Grow the hybridomas in your hybridoma growth medium supplemented with 5% BriClone.
2. Count the cells and dilute in growth medium supplemented with 5% BriClone to a density of 1 cell/100ul.
3. Plate 200ul of caell suspension into each well of a 96 well plate.
4. Let the clones grow undisturbed for 10 days at 37˚C.

Hybridoma Revival

BriClone can be used to increase the viability of hybridomas when thawing from a frozen stock

1. Warm up 10ml of hybridoma growth medium supplemented with 5% BriClone.
2. Take a frozen cryovial and place in a water bath at 37˚C until the ice pellet is nearly thawed.
3. Transfer the cell suspension into the warm 10ml.
4. Centrifuge 5 minutes at 1000rpm.
5. Decant the supernatant and resuspend the cells into an appropriate volume of hybridoma growth medium supplemented with 5% BriClone.
6. Transfer the cells into the desired cell culture vessel.
7. Grow the cells at 37˚C.