

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

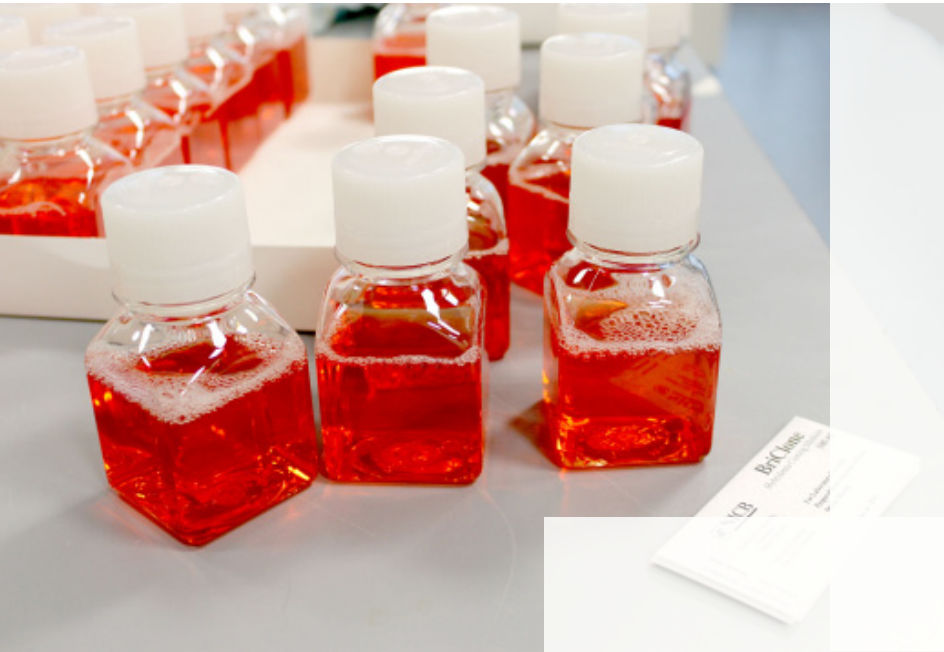
Once thawed, BriClone is stable for 2 weeks at +4°C

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 your 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



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 cell 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