



Instructions for BC3 Cell Line

Description/Overview

Autoimmune T cells are believed to be the proximate cause of multiple sclerosis (MS). T cells specific for myelin antigens cause inflammatory demyelination in animal models of MS and have been extensively studied for decades. Myelin basic protein is the myelin antigen that has been most studied and MBP specific T cells have been detected in patients with MS.

The BC3 cell line was isolated from a normal 56 year old Caucasian man. In vitro stimulation of peripheral blood mononuclear cells (PBMC) with a peptide of MBP was used to select for peptide reactive T cells. The peptide used (ENPVVHFFKNIVTPRTP) is believed to be the immunodominant epitope. The HLA restriction of this cell line is not known but this peptide binds most readily to HLA-DR β 1*1501, an allele expressed by this donor. The cells also recognize the peptide FFKNIVTPRTPPPSQGK, thus the minimal epitope for this line is FFKNIVTPRTP.

The clonality of this cell line has not been determined.

Recommended medium

We recommend X-VIVO™15 medium (Lonza, Walkersville, MD) for culture and maintenance of BC3 cells. Other media may also be adequate but have not been evaluated.

Thawing

The procedure for thawing cells is available at www.astartebio.com/thaw. Briefly, cells should be thawed rapidly in a 37°C water bath and then transferred to a centrifuge tube containing 9 mL of culture medium. The cells can then be centrifuged for 10 min at 200 x g to pellet the cells. Resuspend the cells in recommended culture medium and proceed with experimental manipulations.

Some clumping of the cells may be observed. If clumping is problematic, culture the cells for 1-3 days in not more than 10 U/mL interleukin 2.



Maintenance and Culture

Antigen specific T cells should be restimulated every 10-14 days. Maintenance in IL-2 alone is not recommended as the cells may become unresponsive to antigen.

Materials required

Antigen or mitogen. The peptide antigen may be purchased from AnaSpec (San Jose, CA). Catalog numbers 60977 and 62731 are both recognized by this cell line. Phytohemagglutinin A (PHA) may be used as a non-specific stimulus.

Antigen presenting cells – PBMC or B lymphoblastoid cell lines (B-LCL). Autologous B-LCL are available from Astarte Biologics. Donor 3 was the source of the T cell line.

X-VIVO™ 15 medium

Human IL-2

Mitomycin C or gamma radiation source

24 well tissue culture plates

Equipment

CO₂ incubator, 37°C

Centrifuge

1. To restimulate the cells, wash with fresh medium and suspend in fresh X-VIVO™15 medium at a concentration of $2-5 \times 10^5$ per mL. Aliquot 1 mL of cells per well of a 24 well tissue culture plate.
2. Prepare accessory/antigen presenting cells (APC) by inactivating to prevent cell division. If a gamma irradiation source is used PBMC can be inactivated by exposure to 2000 R while B-LCL require 10,000 R for thorough inactivation. Instructions for mitomycin C inactivation are below.
 - a. Suspend PBMC at 5×10^6 per mL in PBS.
 - b. Add mitomycin C to a final concentration of 25 ug/mL and incubate 30 minutes at 37°C.
 - c. Wash three times to remove mitomycin C.
 - d. If B-LCL are used suspend the cells at 2×10^6 per mL in PBS.
 - e. Add mitomycin C to a final concentration of 40 ug/mL and incubate for 30 minutes at 37°C.
 - f. Wash three times to remove mitomycin C.



If specific peptide is used to stimulate the APC must match the HLA type of the BC3 cell line (see certificate of analysis). If mismatched cells are used, a mitogen such as PHA may be used to stimulate. APC are adjusted to 10^6 /mL if PBMC are used and 2×10^5 per mL if LCL are used. Equal volumes of APC are added to the BC3: 1 mL per well of 24 well plate. Peptide (10 ug/mL) or mitogen is then added and the plate is incubated at 37°C, 5-6% CO₂.

3. After 24 hours, mix each well thoroughly and transfer to a 75 cm² flask. Add 18 mL of fresh medium per well and add IL-2 to achieve a final concentration of 20 U/mL.
4. Incubate for 10-14 days prior to use in an assay or restimulation. If culture medium turns yellow, check for contamination. If no contamination is observed, split culture by mixing and distributing into additional flasks. A shift in the pH of the medium is normal and expected 7-10 days after stimulation. Cells may reach a density of 1-2 million per mL.

Antigen

The peptide antigen may be purchased from AnaSpec (San Jose, CA). Catalog numbers 60977 and 62731 are both recognized by this cell line

Assay

BC3 cells may be used for assay immediately after thawing.

BC3 cells are tested for antigen reactivity by incubating in a 96 well plate with mitomycin C inactivated autologous PBMC or B-LCL and peptide. Inactivated B-LCL serve as antigen presenting cells and are plated at 2×10^4 per well. T cells are plated at 20,000 cells per well and the peptide is added at 1-50 ug/mL. Optimal peptide concentration depends upon the readout of the assay and the condition of the cells. Maximal proliferation is typically observed at lower antigen concentration than cytokine secretion. Culture medium can be collected at 24 hours for analysis of cytokine secretion. Proliferation is typically measured at 3-4 days depending on the method used.

Assay conditions may vary in your laboratory. The parameters given serve as starting points only.