

**INSTRUCTION MANUAL**  
**CORNING<sup>®</sup> BIOCOAT<sup>™</sup> ANTI-HUMAN CD3**  
**T-CELL ACTIVATION PLATES**

**Package Insert for Product Catalog Numbers:**

**354725**

***Note:*** Product should be stored at 2-8°C upon arrival. Product is stable for a minimum of 3 months upon shipment.

Discovery Labware, Inc., Two Oak Park, Bedford, MA 01730, Tel: 1.978.442.2200 (U.S.)  
CLSTechServ@Corning.com                      www.corning.com/lifesciences

**CORNING**

***For Research Use Only. Not for use in diagnostic or therapeutic procedures.***

For a listing of trademarks, visit [www.corning.com/lifesciences/trademarks](http://www.corning.com/lifesciences/trademarks)

© 2013 Corning Incorporated

## **Product Description**

Plate-bound antibodies against the T-cell receptor complex have been used to induce T-cell activation in a variety of species. Corning® BioCoat™ Anti-Human CD3 T-Cell Activation Plates are coated with high quality anti-CD3 antibodies. Available for use with human T cells, Corning BioCoat Anti-Human CD3 T-Cell Activation Plates offer lot-to-lot consistency and come individually packaged with lids for ease of use. All lots are quality tested for the ability to activate T cells in culture.

### **Corning BioCoat Anti-Human CD3 T-Cell Activation Plate Applications include:**

- T-cell Activation
- Cytokine Production
- Cytokine mRNA Quantitation
- Co-stimulation
- Drug Studies on T-cell function

### **Corning BioCoat Anti-Human CD3 T-Cell Activation Plates Quality Control:**

- Tested negative for presence of bacteria and fungi
- Tested for ability to activate Jurkat cells

## **HUMAN PBMC PROLIFERATION ASSAY**

### **CELL CULTURE**

#### **▪ Medium formulation**

- RPMI 1640 with 2 mM L-glutamine, containing 10% FBS

#### Human PBMC Preparation

1. Obtain human blood; add an equal volume of DPBS to the blood sample.
2. Slowly layer the Ficoll-Paque™ PLUS solution (Amersham Pharmacia Biotech AB, 17-1440-02) underneath the blood/PBS mixture. Use 3 mL Ficoll-paque per 10 mL blood/PBS mixture.
3. Centrifuge 30 min at  $900 \times g$ , 18°C to 20°C, with no brake.
4. Using a sterile pipet, remove the upper layer that contains the plasma and most of the platelets. Using another pipet, transfer the mononuclear cell layer to another centrifugation tube.
5. Wash cells twice by adding excess PBS and centrifuge 10 min at  $400 \times g$ .

***For Research Use Only. Not for use in diagnostic or therapeutic procedures.***

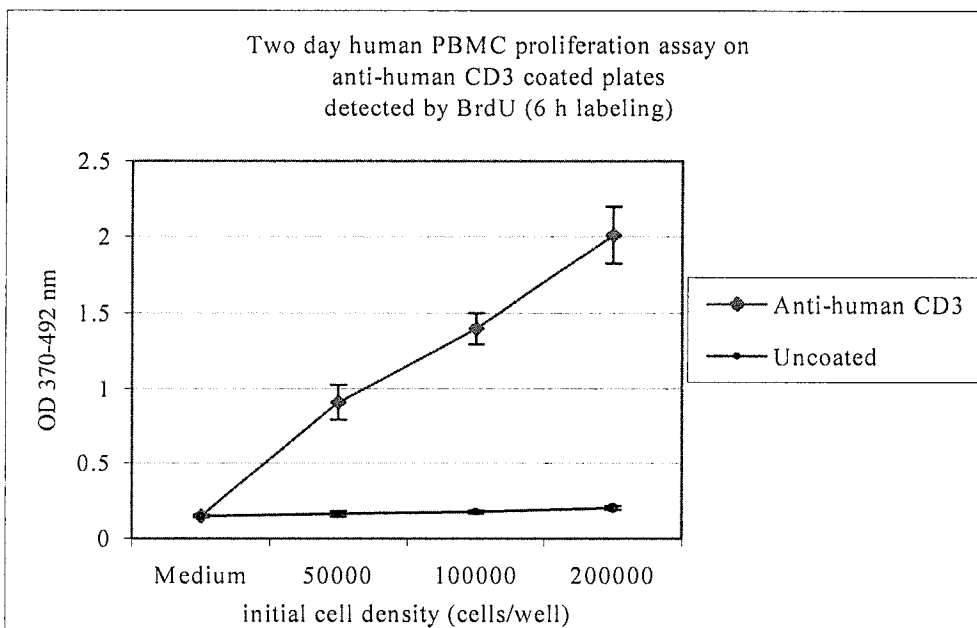
For a listing of trademarks, visit [www.corning.com/lifesciences/trademarks](http://www.corning.com/lifesciences/trademarks)

© 2013 Corning Incorporated

6. Resuspend cells in RPMI medium and count the cells using a Coulter counter.

▪ **Human PBMC proliferation assay**

1. Seed PBMC in desired density ( $1.0 \times 10^4$  to  $2.0 \times 10^5$  cells/well) into anti-human CD3 plates and place in CO<sub>2</sub> incubator at 37°C for 45 to 48 hours.
2. Detect cell proliferation using Cell Proliferation ELISA BrdU Kit (Roche, 1 647 229). Briefly, label cells for 2 h to 24 h in CO<sub>2</sub> incubator at 37°C; remove medium, fix the cells and denature the DNA; remove fixing/denature agent, add anti-BrdU-POD and incubate for 90 min at RT; wash cells three times, then add substrate solution and incubate for 10 min at RT; read absorption at 370 nm with 492 nm as reference wavelength.



**ACTIVATION-INDUCED CELL DEATH ASSAY OF HUMAN JURKAT CELLS**

**CELL CULTURE**

- **Medium formulation** per 500 mL RPMI-1640 with 10% fetal bovine serum:
- HEPES (1M): 10 mL
  - L-glutamine (200 mM): 5 mL
  - Sodium pyruvate (100 mM): 5 mL
  - Gentimicin sulfate (50 mg/mL): 0.6 mL

*For Research Use Only. Not for use in diagnostic or therapeutic procedures.*

For a listing of trademarks, visit [www.corning.com/lifesciences/trademarks](http://www.corning.com/lifesciences/trademarks)

© 2013 Corning Incorporated

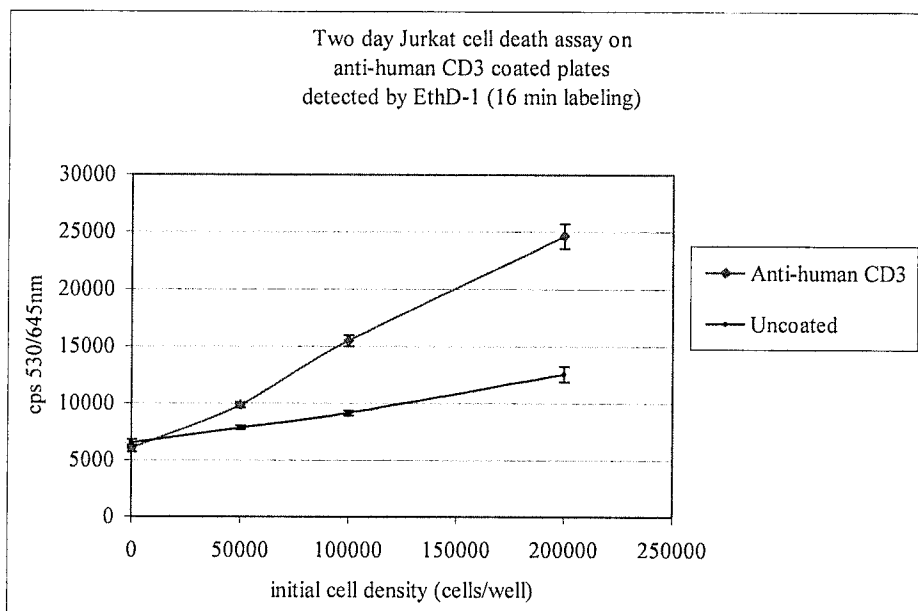
## ▪ Jurkat Cell Preparation

Jurkat E6-1 - Use cells which have been passed for at least one day, and which are below passage number 30. Explicitly follow the ATCC product information sheet concerning cell density.

1. Harvest the Jurkat cells and spin for 5 min at  $200 \times g$ .
2. Decant or aspirate the medium from the tube(s) and combine the cells into one tube with 50 ml of DPBS in order to wash the cells.
3. Spin the cells for 5 min at  $200 \times g$  and decant the supernatant.
4. Resuspend cells at desired concentration (in this case,  $2 \times 10^6$  cells/mL).

## Activation-induced Jurkat cell death assay

1. Seed the cells in desired cell density ( $0.5 \times 10^5$  to  $2.0 \times 10^5$  cells/well) into anti-human CD3 plates and uncoated (control) plates, 100  $\mu$ L/well.
2. Incubate in a 5% CO<sub>2</sub> incubator @ 37°C for 45 to 48 hours.
3. Dissolve ethidium homodimer-1 powder (EthD-1, Molecular Probes, E-1169) in 20% DMSO/H<sub>2</sub>O (v/v) to make a 2 mM stock solution, aliquot, and store the aliquots at  $-20^\circ\text{C}$ .
4. Dilute the EthD-1 stock solution in DPBS to make a 10  $\mu$ M working solution.
5. To each well add 100  $\mu$ L of EthD-1 working solution, and read the plates in the Wallac Victor<sup>2</sup> fluorescence plate reader using 530 nm excitation and 645 nm emission filters.
5. The EthD-1 penetrates non-living cells and its fluorescence is increased. At  $2 \times 10^5$  cells/well the signal to noise ratio (compared to cells cultured on an uncoated plate) should be greater than 2.5.



**For Research Use Only. Not for use in diagnostic or therapeutic procedures.**

For a listing of trademarks, visit [www.corning.com/lifesciences/trademarks](http://www.corning.com/lifesciences/trademarks)

© 2013 Corning Incorporated

SAFETY RECOMMENDATION: Handle in accordance with good industrial hygiene and laboratory safety practices

## Ordering Information:

Antibody Coating	Plate Configuration	Shelf-Pack Catalog Number (5 per pack)
Anti-Human CD3	96 well, clear with lid	354725
Uncoated Control	96 well, clear with lid	354730

## Related Products:

### Corning® BioCoat™ Products

Antibody Coating	Plate Configuration	Shelf-Pack Catalog Number (5 per pack)
Anti-Mouse CD3	96 well, clear with lid	354720

### Falcon® Products

Product	Description	Ordering Information
Falcon 50 ml Conical Tube	Polypropylene tube, 25/rack, sterile	352098 (500/cs)
Falcon 100 mm Petri Dish, Standard	100 mm x 15 mm, sterile, standard style	351029 (500/cs)
Falcon 5 ml Pipet	Serological pipet, individually wrapped	357543 (50/bag)
Falcon 10 ml Pipet	Serological pipet, individually wrapped	357551 (50/bag)

***For Research Use Only. Not for use in diagnostic or therapeutic procedures.***

For a listing of trademarks, visit [www.corning.com/lifesciences/trademarks](http://www.corning.com/lifesciences/trademarks)

© 2013 Corning Incorporated

## Corning® Discovery Labware Products

Product	Description	Ordering Information
IL-2 Human Natural	Tested for ability to stimulate proliferation of the mouse T-Cell line CTLL.	354121 (2,000 BRMP Units)
IL-2 Human Recombinant	Tested for ability to stimulate proliferation of the mouse T-Cell line CTLL.	354043 (10,000 BRMP Units) 356043 (50,000 BRMP Units)

**For Technical Service Information, please contact Technical Support:  
1.800.492.1110; CLSTechServ@corning.com**

Discovery Labware, Inc., Two Oak Park, Bedford, MA 01730, Tel: 1.978.442.2200 (U.S.)  
CLSTechServ@Corning.com                      www.corning.com/lifesciences

**CORNING**

***For Research Use Only. Not for use in diagnostic or therapeutic procedures.***

For a listing of trademarks, visit [www.corning.com/lifesciences/trademarks](http://www.corning.com/lifesciences/trademarks)  
© 2013 Corning Incorporated