

PBMC Based T Cell Proliferation Assay

Instructions for use

Safety statements

THESE PRODUCTS ARE FOR RESEARCH USE ONLY. Not approved for human or veterinary use, for application to humans or animals, or for use in clinical or *in vitro* procedures.

WARNING: LONZA PRIMARY CELLS CONTAIN HUMAN SOURCE MATERIAL; TREAT AS POTENTIALLY INFECTIOUS. Each donor is tested and found non-reactive by an FDA-approved method for the presence of HIV-1, hepatitis B virus and hepatitis C virus. Where donor testing is not possible, cell products are tested for the presence of viral nucleic acid from HIV-1, hepatitis B virus, and hepatitis C virus. Testing cannot offer complete assurance that HIV-1, hepatitis B virus, and hepatitis C virus are absent. All human-sourced products should be handled at the biological safety level 2 to minimize exposure to potentially infectious products, as recommended in the CDC-NIH manual, [Biosafety in Microbiological and Biomedical Laboratories](#), 5th edition. If you require further information, please contact your site safety officer or Scientific Support.

Preparation of reagents for T cell assay

All work should be done in a laminar flow hood. Decontaminate the external surfaces of all supplement vials and the medium bottles with ethanol or isopropanol.

1. IL-2 Medium

Make IL-2 media by adding 1 μ L of 5 μ g/mL IL-2 working solution to 1 mL X-VIVO® 15 Serum-free Hematopoietic Cell Medium for a final concentration of 5 ng/mL IL-2.

2. CFSE reagent

Follow company protocols for stock and working solution of CFSE stain. Working solution should be at final concentration of 5 μ M.

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NOTE: All work is to be performed in a laminar flow hood. After addition of CFSE, work should be protected from light.

1. Pipette 10 mL of pre-warmed X-VIVO® 15 Serum-free Hematopoietic Cell Medium into 15 mL conical tube and place in laminar flow hood.
2. Thaw PBMCs at 37°C for ~2 min until only a sliver of ice remains and pipette cells into X-VIVO® 15 Serum-free Hematopoietic Cell Medium in 15 mL conical from step 1.
3. Wash cell vial with 1 mL X-VIVO® 15 Serum-free Hematopoietic Cell Medium from conical and add back to conical.
4. Centrifuge cells at 300xg for 10 minutes at 4°C
5. Resuspend pellet in 1 mL X-VIVO® 15 Serum-free Hematopoietic Cell Medium and count using Trypan Blue and a hemocytometer.
6. After counting, remove the needed number of cells and place them into a new 50 mL conical. For example, one reaction requires 1×10^6 cells/mL. Adjust according to number of reactions and reaction volume.
7. Add 1 mL CFSE 5 μ M working solution per 1×10^6 cells and mix by vortexing
8. Incubate for 20 minutes protected from light at room temperature.
9. After incubation, add X-VIVO® 15 Serum-free Hematopoietic Cell Medium at 4X the volume as CFSE stain to stop the reaction.
10. Centrifuge cells 300xg for 10 minutes at room temperature.
11. Resuspend cells in X-VIVO® 15 Serum-free Hematopoietic Cell Medium at a concentration of 10×10^6 cells/mL.
12. Incubate cells at room temperature for 10 minutes.
13. During incubation step, prepare tissue culture plates. For a 24-well plate, the following volumes are recommended:
 - a. **Negative control wells:** 900 μ L X-VIVO® 15 Serum-free Hematopoietic Cell Medium
 - b. **IL-2 control wells:** 900 μ L IL-2 Medium
 - c. **Positive control wells:** 850 μ L IL-2 Medium + 50 μ L CD3/CD28 DynaBeads (ThermoFisher 11131D)
14. After the 10 minute incubation from step 12, add cells to each well (final concentration of 1×10^6 cells/mL – adjust according to tissue plate size). For 24 well plates, add 100 μ L cells.
15. Allow assay to incubate for 4 days at 37°C, 5% CO₂ to allow for T cell proliferation.

16. On day 4, collect T cell suspension from cultures and stain with Live/Dead fixable cell dye for 20 minutes and analyze via FACS. A recommended minimum of 100,000 cells for FACS analysis should be taken into consideration.
17. Assess cells for CFSE intensity, decreased CFSE levels indicates increased T cell proliferation. .

Ordering information

Catalog no.	Description	Size
CC-2702	Human Peripheral Blood Mononuclear Cells (hPBMC), Cryopreserved	≥50 million cells
04-418Q	X-VIVO® 15 Serum -free Hematopoietic Cell Medium	1L, complete with L-Glutamine, gentamicin, and phenol red, xenofree

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IL-2 (R&D Systems 202-IL-500) mentioned is a product from R&D Systems.

Dynabeads (ThermoFisher 11131D) mentioned is a product of ThermoFisher Scientific.

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