Store at 4°C

Rapid-Act T Cell Activation Kit (Human, Anti-CD3/CD28)

#88179

1 Kit
(50 assays)

Cell Signaling TECHNOLOGY®

Support: +1-978-867-2388 (U.S.)
www.cellsignal.com/support

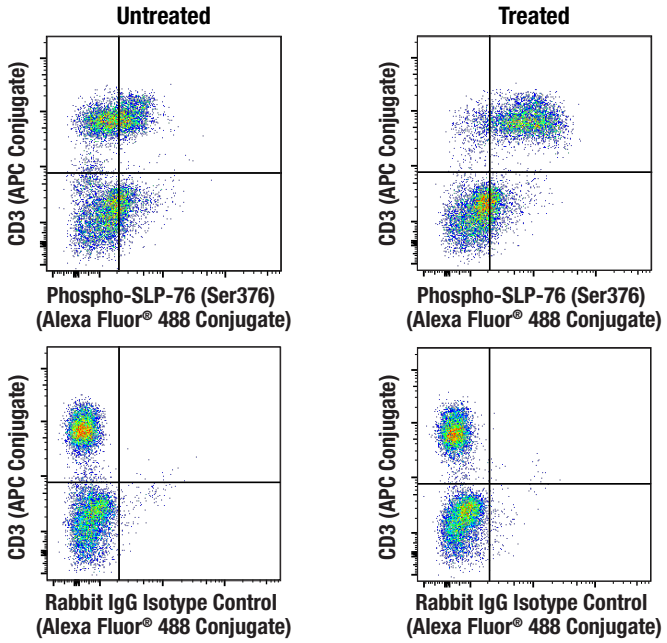
Orders: 877-616-2355 (U.S.)
orders@cellsignal.com

For Research Use Only. Not For Use In Diagnostic Procedures.

Product Includes	Product #	Kit Quantity	Storage Temp
Rapid-Act Anti-human CD3 Mouse mAb	43737	125 µg	4°C
Rapid-Act Anti-human CD28 Mouse mAb	58489	125 µg	4°C
Rapid-Act Synthetic Immune Synapse	68999	500 µL	4°C

Description: The Rapid-Act T Cell Activation Kit (Human, Anti-CD3/CD28) provides the necessary reagents for easy, single-step induction of human T cell activation and proliferation. The included Synthetic Immune Synapse particles pre-cluster the CD3 and CD28 antibodies for thorough T cell activation, and are small enough to minimize cell clumping. Because the reagents are added to the cells in a single step, the kit enables accurate time course studies for short-term T cell stimulation. The kit can also be used for longer-term T cell proliferation and exhaustion assays. No modification or removal is necessary prior to subsequent analysis by flow cytometry.

Background: T cells are activated by signaling through ITAM (immunoreceptor tyrosine-based activation motif)-containing CD3 signaling chains that associate with the T cell receptor (1). Co-stimulation through CD28 on T cells provides an additional signal required for effective T cell activation (2). Antibody-based binding and aggregation of CD3 and CD28 proteins simulates the engagement of a T cell with an antigen presenting cell, and results in T cell activation.



Flow cytometric analysis of human peripheral blood mononuclear cells, untreated (left column) or treated with Rapid-Act T Cell Activation Kit (Human, Anti-CD3/CD28) (15 min; right column), using Phospho-SLP-76 (Ser376) (E3G9U) XP® Rabbit mAb (Alexa Fluor® 488 Conjugate) #47876 (top row) or concentration-matched Rabbit (DA1E) mAb IgG XP® Isotype Control (Alexa Fluor® 488 Conjugate) #2975 (bottom row), and co-stained with CD3 (UCHT1) Mouse mAb (APC Conjugate) #19881.

Storage: Store at 4ºC. All components in this kit are stable for 12 months when kept in the original format and stored at 4ºC. Once reconstituted, store the Anti-human CD3 and Anti-human CD28 at -80ºC. *Aliquot to avoid multiple freeze/thaw cycles.*

Background References:

(1) Pitcher, L.A. and van Oers, N.S. (2003) *Trends Immunol* 24, 554-60.

(2) June, C.H. et al. (1990) *Immunol Today* 11, 211-6.

Thank you for your recent purchase. If you would like to provide a review visit cellsignal.com/comments.

Applications: W—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **FC-FP**— Flow cytometry-Fixed/Permeabilized **FC-L**— Flow cytometry-Live **E-P**—ELISA-Peptide
Species Cross-Reactivity: H—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse
All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

#88179

Rapid-Act T Cell Activation Kit (Human, Anti-CD3/CD28) Protocol

A. Solutions and Reagents

Supplied Reagents

1. Rapid-Act Anti-human CD3 Mouse mAb, lyophilized, 125 µg (#43737)
2. Rapid-Act Anti-human CD28 Mouse mAb, lyophilized, 125 µg (#58489)
3. Rapid-Act Synthetic Immune Synapse, 500 µL (# 68999)

Additional Reagents (Not Supplied)

1. 1X Phosphate Buffered Saline (PBS): To prepare 1 L 1X PBS: add 100 mL 10X PBS (#12528) to 900 mL reverse osmosis deionized (RODI) or equivalent grade water
2. Cell Culture Medium (assay dependent)

B. Reagent Preparation

NOTE: Volumes are suitable for stimulation and expansion of 1 x 10^6 cells. For different cell numbers, adjust volumes according to Table 1.

NOTE: We recommend preparing the Rapid-Act CD3/CD28 complex fresh for each experiment. If necessary, store pre-mixed components at 4°C. Do not freeze.

1. Dissolve each Rapid-Act antibody in 500 µL 1X PBS. Store Rapid-Act antibody suspensions in aliquots at -80°C.
2. In a microcentrifuge tube, combine 10 µL Rapid-Act Anti-human CD3 with 10 µL Rapid-Act Anti-human CD28. Mix well. Add 10 µl of Rapid-Act Synthetic Immune Synapse and mix well. Incubate the mixture for at least 20 min at 4°C to generate Rapid-Act CD3/CD28 complex.

C. Cell Preparation

1. Collect cells of interest and pellet by centrifugation. PBMCs, enriched T cells, or cell lines may be used. Recommended cell numbers and volumes in every step are identical to pre-isolated T cells.
2. Resuspend cells in cell culture medium at a concentration of 0.2 x 10^6 - 1 x 10^6 cells per 1 mL (optimal conditions should be titrated).

Table 1: Recommended volumes for different cell numbers

	96-well	48-well	24-well
Cell number	5 – 8 x 10^4	2 – 5 x 10^5	0.5 – 1 x 10^6
Cell culture medium (mL)	0.1 – 0.2	0.5 – 1.0	1.0 – 2.0
Rapid-Act CD3/CD28 complex (µL)	3	15	30

D. T cell Activation and Expansion Procedure

T cell activation

1. Seed 0.5 – 1 x 10^6 cells in 1 – 2 mL cell culture medium (or use volumes and cell densities as determined in Table 1).
2. Add 30 µL Rapid-Act CD3/CD28 complex to the cells and mix gently. Incubate cell suspension in a humidified CO_2 incubator at 37°C, according to your experimental setup.
3. Harvest activated T cells, wash twice with excess 1X PBS by centrifugation, and use directly for further analysis.

T cell expansion

NOTE: Activation markers CD25 and CD69 should be upregulated after 48 h.

1. Activate T cells following steps 1 and 2 in T cell activation section above.
2. Examine culture daily, observing cell size, shape, and cluster formation using a microscope. Count the cells at least every 2 – 3 days to evaluate cell density; do not exceed 2.5 x 10^6 cells/mL. If the cell medium turns yellow or cell density is too high, split cultures back to a density of 0.2 – 1 x 10^6 cells/mL into a new container of appropriate size.
3. Restimulation of the cells might be necessary after 2 – 3 days in culture, with signs of exhaustion typical after 7 – 10 days. Repeat addition of Rapid-Act CD3/CD28 complex as described in T cell activation to restimulate cells.
4. Harvest expanded T cells, wash twice with excess 1X PBS by centrifugation, and use directly for further analysis.