

Store at
4°C

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

#86772

1 Kit
(50 assays)

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

Product Includes	Product #	Kit Quantity	Storage Temp
Rapid-Act Anti-mouse CD3 Hamster mAb	46345	125 µg	4°C
Rapid-Act Anti-mouse CD28 Mouse mAb	63606	125 µg	4°C
Rapid-Act Synthetic Immune Synapse	68999	500 µL	4°C

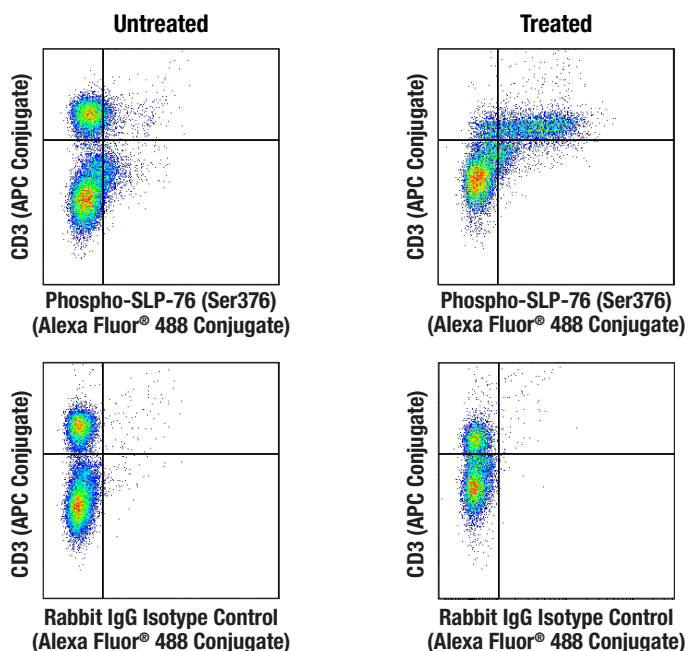
Description: The Rapid-Act T Cell Activation Kit (Mouse, Anti-CD3/CD28) provides the necessary reagents for easy, single-step induction of mouse T cell activation and proliferation. The included Synthetic Immune Synapse particles pre-cluster the CD3 and CD28 antibodies for thorough T cell activation, 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.

Storage: Store at 4°C. All components in this kit are stable for 12 months when kept in the original format and stored at 4°C. Once reconstituted, store the Anti-mouse CD3 and Anti-mouse CD28 at -80°C. *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.



Flow cytometric analysis of mouse splenocytes, untreated (left column) or treated with Rapid-Act T Cell Activation Kit (Mouse, 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 (17A2) Rat mAb (APC Conjugate) #24265.

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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.

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

A. Solutions and Reagents

Supplied Reagents

1. Rapid-Act Anti-mouse CD3 Hamster mAb, lyophilized, 125 µg (#46345)
2. Rapid-Act Anti-mouse CD28 Mouse mAb, lyophilized, 125 µg (#63606)
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 1×10^6 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-mouse CD3 with 10 µL Rapid-Act Anti-mouse 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. Splenocytes, 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 $0.2 \times 10^6 - 1 \times 10^6$ cells per 1 mL (optimal conditions should be titrated).

D. T Cell Activation and Expansion Procedure

T CELL ACTIVATION

1. Seed $0.5 - 1 \times 10^6$ 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 CO₂ 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 2.5×10^6 cells/mL. If the cell medium turns yellow or cell density is too high, split cultures back to a density of $0.2 - 1 \times 10^6$ 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.

Table 1: Recommended volumes for different cell numbers

	96-well	48-well	24-well
Cell number	$5 - 8 \times 10^4$	$2 - 5 \times 10^5$	$0.5 - 1 \times 10^6$
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