Bystander Receptor Blockade: IFNλR1, TNFRSF1A, ICAM-1 and FAS

Sandra L. Ross, Marika Sherman, Patricia L. McElroy, Julie A. Lofgren, Gordon Moody, Patrick A. Baeuerle, Angela Coxon, Tara Arvedson

Abstract

This protocol describes the use of neutralizing antibodies to block receptrs for assessing involvement in bystander killing by BiTE®-activated T cells.

Citation: Sandra L. Ross, Marika Sherman, Patricia L. McElroy, Julie A. Lofgren, Gordon Moody, Patrick A. Baeuerle, Angela Coxon, Tara Arvedson Bystander Receptor Blockade: IFNλR1, TNFRSF1A, ICAM-1 and FAS. **protocols.io**
dx.doi.org/10.17504/protocols.io.hw8b7hw
Published: 17 May 2017

Protocol

Assemble Materials

Step 1.

Materials	Company	Cat.no.
ViewPlate-96 black plate Packardview plate (imaging)	Perkin Elmer	6005182
Effector cells – unstimulated pan T cells	AllCells	PB009-1F
Target cells – SW620, NUGC4	Amgen cell bank	-
BiTE®s – EGFR, MEC14	Amgen	-
CellStripper™ Dissociation Reagent nonenzymatic 1X	Corning	25056Cl
1x PBS	Gibco	14190
50 ml Falcon Tube	BD	35 2070
15 ml Falcon Tube	BD	35 2096
Growth medium: RPMI 1640 medium Supplements: 100U/ml penicillin/streptomycin 10% heat-inactivated fetal bovine serum (FBS)	Gibco Gibco Gibco	11875-093 15140-122 10082-147
Assay medium: RPMI 1640 medium Supplements: 1x nonessential amino acids (NEAA) 10mM HEPES 50µM 2-ß-mercaptoethanol 1mM sodium pyruvate 100U/ml penicillin/streptomycin 5% heat-inactivated fetal bovine serum (FBS)	Biochrom Gibco Gibco Sigma Gibco Gibco Gibco	FG1215 11140-050 15630-080 M6250 11360-070 15140-122 10082-147
Recombinant IFNλ	Roche	11040596001
Recombinant TNFα	Roche	11371843001
Hoechst 33342 nuclear dye	ThermoFisher	62249
Formaldeyde 16% (w/v) methanol-free	Pierce/ThermoFisher	28908
Mouse anti-human ICAM-1 neutralizing antibody (Clone 84H10)	Beckman Coulter	IM0544
Mouse anti-human FAS neutralizing antibody (Clone ZB4)	Enzo	227-E
Mouse anti-human FAS activating antibody (Clone CH11)	Millipore	05-201
Mouse anti-human IFNλR1 (Clone GIR208)	R&D Systems	MAB6732
Mouse anti-human IFNλR1 (Clone 92101)	R&D Systems	MAB6731

Mouse anti-human TNFRSF1A (Clone 16803)	R&D Systems	MAB225
Control mouse antibody muIgG1 (clone NCG01)	Abcam	ab81032

Prepare BiTE®-activated and control T cells in bulk

Step 2.

Day 1: Combine $EGFR^{+}$ (NUGC4) + T cells (10:1 E:T) +/- 100 pM EGFR BiTE® in T75 flask. Please refer to BiTE® bystander T cell dependent cellular cytotoxicity (TDCC) assay protocol (Step 5 – TDCC assay using BiTE®-activated T cells).

Plate target cells

Step 3.

Day 1: Prepare $EGFR^{-}$ (SW620) cells by plating 10,000 cells/well in ViewPlate-96 black plates. Please refer to BiTE® T cell-dependent cellular cytotoxicity (TDCC) assay protocol (Step 5 – prepare target cells). If blocking ICAM-1 or FAS, treat cells +/- 10ng/ml IFNγ and 5ng/ml TNFα for 24 hours.

Harvest BiTE®-activated and control T cells

Step 4.

Day 2: Harvest bulk BiTE®-activated and control T cells prepared in step 2. Please refer to BiTE® bystander T cell dependent cellular cytotoxicity (TDCC) assay protocol (Step 5 – TDCC assay using BiTE®-activated T cells). Resuspend cells at 2x10e6 cells/ml (will use 50 µl/well = 100,000 cells/well)

Prepare 2X blocking antibody solutions in Assay Medium

Step 5.

Day 2:

Antibody	Clone #	Final Conc.	2X Conc.
Anti-FAS blocking	ZB4	2.5 µg/ml	5 µg/ml
Anti-ICAM-1 blocking	84H10	5 µg/ml	10 µg/ml
Anti-IFNλR1 blocking	GIR208	2 µg/ml	4 µg/ml
Anti-IFNλR1 blocking	92101	2 µg/ml	4 µg/ml
Anti-TNFRSF1A blocking	16803	2 µg/ml	4 µg/ml
Control antibody	NCG01	2, 2.5, 5 µg/ml	4, 5, 10 µg/ml
Anti-FAS activating	CH11	2.5 µg/ml	5 µg/ml

Set up assay

Step 6.

Day 2:

1. Remove medium from all wells
2. Add 50 µl/well 2X control or blocking antibody to designated wells; incubate for 1 hour at 37°C
3. Add 50 µl/well control or activated T cells prepared in Step 4 to designated wells (for E:T of 10:1, use 2x10e6 cells/ml; 50 µl = 100,000 cells/well). Note: BiTE® dilutions can be added to T cells prior to adding to assay plate.
4. As a control for FAS activation, add 50 µl/well Assay Medium + 50 µl/well 2X FAS activating antibody to designated wells
5. Incubate plates for 24 hours at 37°C

Measure cytotoxicity

Step 7.

Day 3: Fix cells, stain nuclei with Hoechst nuclear dye and count 16 10X fields on ArrayScan using Target Activation bioapplication. Please refer to BiTE® T cell-dependent cellular cytotoxicity (TDCC) assay protocol (Step 8 – Cytotoxicity assay).