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# Whole blood T cell assay for NHPs, containment protocol

Y Forked from <u>Immunophenotyping for NHPs, containment</u> protocol

COMMENTS 0

DOI

# dx.doi.org/10.17504/protocols.io.bp2l692prlqe/v1

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WORKS FOR ME 1



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DISCLAIMER

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### **ABSTRACT**

This is a protocol used to perform T cell assays using whole blood (collected in EDTA tubes) or cells from bronchoalveolar lavage (BAL). We have successfully used this protocol for rhesus macaques, cynomolgus macaques, and African green monkeys (although antibody mix provided was titrated on macaques). It allows the characterization of T cells into Th1 and Th2 (or Tc1 and Tc2) phenotypes. It technically offers 64 different activation profiles (4 cytokines + CD107a + CD69).

The nature and concentration of the stimulant is left unspecified as we have used different ones depending on reagent availability.

This protocol was designed to deal with samples coming from containment labs (> CL2) at our facility. If you are using this protocol at a different facility please ensure that proper testing and approvals are in place. The protocol can be used for experiments completed entirely in CL2, simply go from step 17 directly to 31.

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PROTOCOL CITATION

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FORK NOTE



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**FORK FROM** 

# Forked from Immunophenotyping for NHPs, containment protocol, Jonathan Audet

**KEYWORDS** 

flow cytometry, nonhuman primate, whole blood, broncho-alveolar lavage, T cell assay

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**GUIDELINES** 

For BAL fluid, make sure to set FSC voltage much lower than for whole blood.

### MATERIALS TEXT

₩ Human TruStain FcX BioLegend Catalog #422302 Step 8

BD FACS Lysing Solution (10X) BD Biosciences Catalog #349202 Step 7

BD Cytofix/Cytoperm BD Biosciences Catalog #554722 In 2 steps

BD Perm/Wash buffer **BD Biosciences Catalog #554723** In 3 steps

Step 10 Step 10

# **Equipment**

FACS Tube	NAME
Tube	TYPE
Falcon	BRAND
14-959-2A	SKU

# Equipment

2 ml screw-cap tubes	NAME
Microtubes	TYPE
Sarstedt	BRAND
72.694.006	SKU

SAFETY WARNINGS

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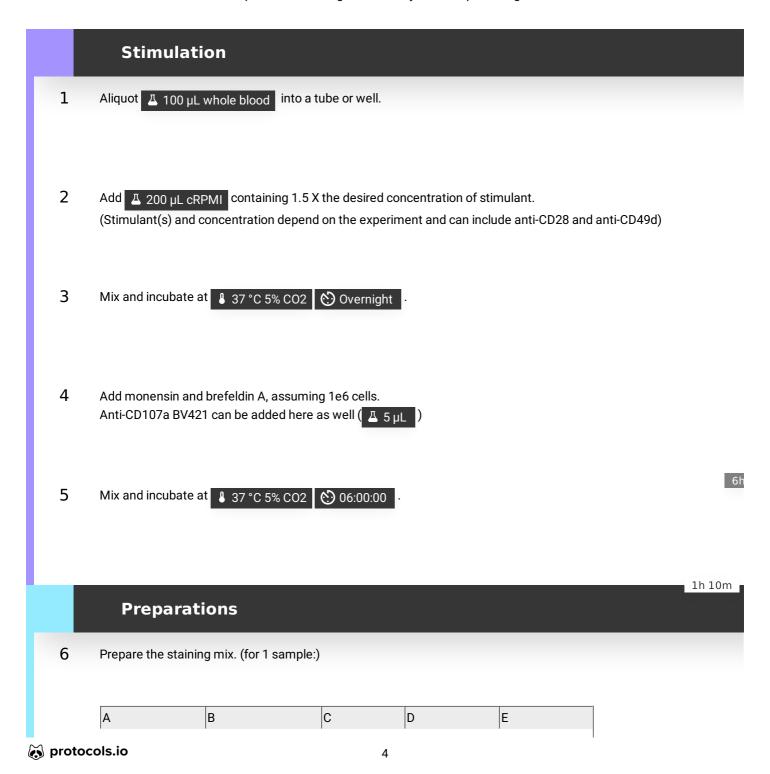
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# **BEFORE STARTING**

The antibody mix described in the methods was tested on Cynomolgus macaque whole blood and BAL fluid. All antibodies should cross-react with rhesus macaques and African green monkeys but the panel might need to be re-titrated.

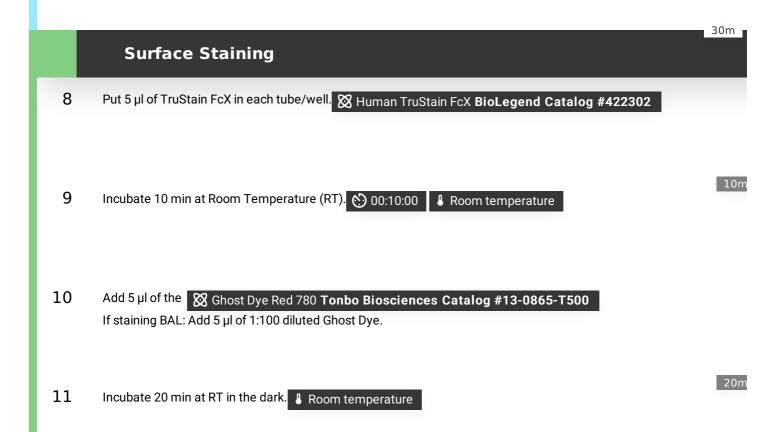


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A	В	С	D	E
Supplier	Antibody	Clone	Channel	Volume per test
BD Biosciences	CD3	SP34-2	Alexa Fluor 700	5
BD Biosciences	CD8	RPA-T8	BV786	5
BD Biosciences	CD45RA	5H9	PE-CF594	2.5
BD Biosciences	CD4	L200	PerCP-Cy5.5	20
BD Biosciences	CCR7	G043H7	BV711	5
BD Biosciences	CD69	FN50	BV650	5
	Total			42.5
	BD Brilliant Stain			57.5

Prepare the 1X FACS Lysing solution by diluting the 10X stock with Milli-Q water. You will need 2 ml of 1X solution for each sample.

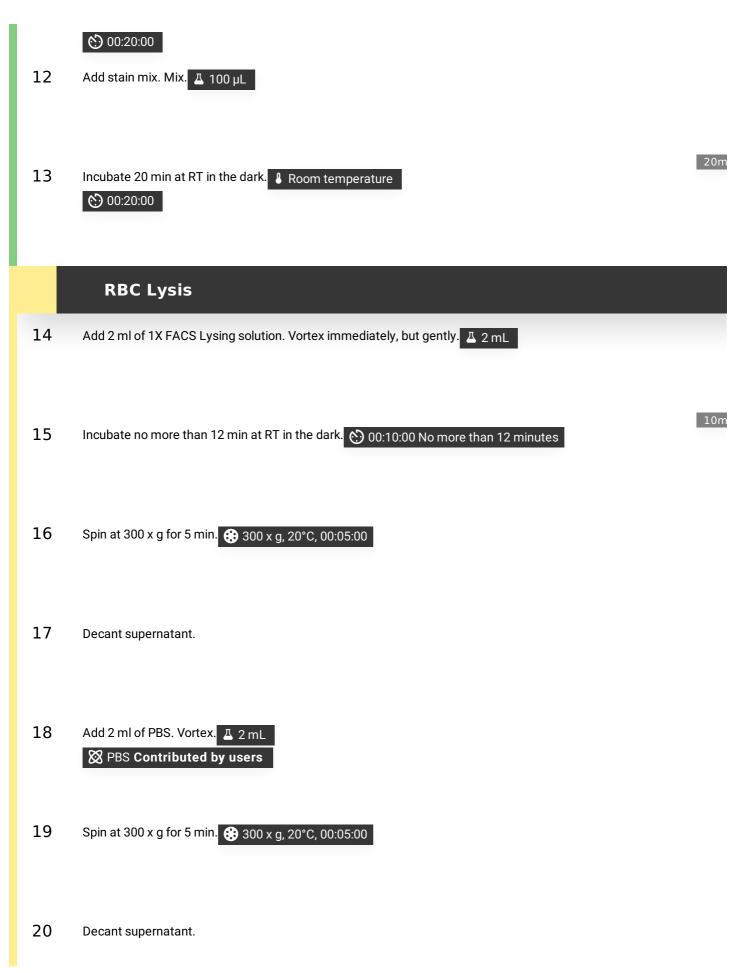
**⋈** BD FACS Lysing Solution (10X) **BD Biosciences Catalog #349202** 



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6

30m

# **Sample Inactivation**

- Resuspend in Cytofix/Cytoperm. 

  BD Cytofix/Cytoperm BD Biosciences Catalog #554722
- 21.1  $100 \, \mu l \, per \, 5 \, x \, 10^5 \, cells.$
- 21.2 Use at least 400 µl for easy decanting.
- 22 Incubate at least 30 min at RT in the dark. 00:30:00 Room temperature In the dark.
- Spin at 500 x g for 8-10 min. 500 x g, 20°C, 00:08:00
- On a clean bench, decant supernatant.
- Use same volume of Cytofix/Cytoperm as before to resuspend the cells.

  BD Cytofix/Cytoperm BD Biosciences Catalog #554722
- Transfer in a 2 ml screwcap tube. Shake the tube to cover all surfaces with Cytofix/Cytoperm.

Equipment	
2 ml screw-cap tubes	NAME
Microtubes	TYPE
Sarstedt	BRAND
72.694.006	SKU

30m

- Transfer tubes from containment space to CL2 space according to the facility's approved protocols/SOPs.
- Tubes can be opened in CL2 (in a BSC) no less than 30 min after the resuspension (step 17). (Samples are generally processed the next day; keep at 4 C overnight, in the dark)

# Intracellular staining Give tubes a quick spin in a tabletop centrifuge. Ensure all tubes have at least a few hundred microliters of Cytofix/Cytoperm. Transfer the samples into 1 ml of BD ⊗ BD Perm/Wash buffer BD Biosciences Catalog #554723 in FACS tubes. Spin at 500 x g for 8-10 min. ♦ 500 x g, 20°C, 00:08:00



Decant supernatant.

33

- Repeat steps 31-33.
- Prepare the staining mix. (for 1 sample:)
  (uses BD Perm/Wash buffer BD Biosciences Catalog #554723 )

A	В	С	D	E
Supplier	Antibody	Clone	Channel	Volume per test
BioLegend	IFNgamma	B27	APC	2.5
BioLegend	TNF	MAb11	PE-Cy7	5
BioLegend	IL-2	MQ1-17H12	Alexa Fluor 488	0.625
BioLegend	IL-4	8D4-8	PE	2.5
	Total			10.625
	PermWash			89.375

- $36 \qquad \text{Resuspend in } 100\,\mu\text{I of intracellular staining mix}.$
- 37 Incubate 4 °C © 00:30:00
- Add 🗸 1 mL 🛭 SBD Perm/Wash buffer BD Biosciences Catalog #554723
- 39 § 500 x g, 4°C, 00:08:00

8m

30m

40	Decant supernatant and blot tubes/plate.
41	Repeat steps 38-40.
42	Resuspend in PBS 1% formaldehyde.
43	Run on flow cytometer.
44	Gating strategy for whole blood: