

Nov 21, 2020 Version 2

PBMC- 02 - CD4+ T cell Isolation from PBMC with "Dynabeads CD4 Positive Isolation Kit" V.2

DOI

dx.doi.org/10.17504/protocols.io.bpxqmpmw

Marco Cosentino¹, Elisa Storelli¹, Alessandra Luini¹, Massimiliano LM Legnaro¹, Emanuela Rasini¹, Marco Ferrari¹, Franca Marino¹

¹Center for Research in Medical Pharmacology, University of Insubria (Varese, Italy)



Farmacologia Medica

OPEN  ACCESS



DOI: dx.doi.org/10.17504/protocols.io.bpxqmpmw

Protocol Citation: Marco Cosentino, Elisa Storelli, Alessandra Luini, Massimiliano LM Legnaro, Emanuela Rasini, Marco Ferrari, Franca Marino 2020. PBMC- 02 - CD4+ T cell Isolation from PBMC with "Dynabeads CD4 Positive Isolation Kit". **protocols.io** <https://dx.doi.org/10.17504/protocols.io.bpxqmpmw> Version created by **Farmacologia Medica**

License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: November 21, 2020

Last Modified: November 21, 2020

Protocol Integer ID: 44752

Abstract

List of published works using this protocol:

- Kustrimovic N., Comi C., Magistrelli L., Rasini E., Legnaro M., Bombelli R., Aleksic I., Blandini F., Minafra B., Riboldazzi G., Struchio A., Mauri M., Bono G., Marino F., Cosentino M. Parkinson's disease patients have a complex phenotypic and functional Th1 bias: cross-sectional studies of CD4+ Th1/Th2/T17 and Treg in drug-naïve and drug-treated patients (2018). Journal of neuroinflammation, 15(1), 205. <https://doi.org/10.1186/s12974-018-1248-8>

- Kustrimovic, N., Rasini, E., Legnaro, M., Bombelli, R., Aleksic, I., Blandini, F., Comi, C., Mauri, M., Minafra, B., Riboldazzi, G., Sanchez-Guajardo, V., Marino, F., & Cosentino, M. (2016). Dopaminergic Receptors on CD4+ T Naive and Memory Lymphocytes Correlate with Motor Impairment in Patients with Parkinson's Disease. Scientific reports, 6, 33738. <https://doi.org/10.1038/srep33738>

- Cosentino M., Ferrari M., Kustrimovic N., Rasini E., Marino F. (2015). Influence of dopamine receptor gene polymorphisms on circulating T lymphocytes: A pilot study in healthy subjects. Human immunology, 76, 10, 747-752. <https://doi.org/10.1016/j.humimm.2015.09.032>

Materials

MATERIALS

 Dynabeads® CD4 Positive Isolation Kit **Thermo Fisher Catalog #11331D**

 Fetal Bovine Serum (FBS) **EuroClone Catalog #ECS0180L-500 ml**

 RPMI 1640 **EuroClone Catalog #ECM 0495L- 500 ml**

 BSA **Sigma Aldrich Catalog #A2153**

 BD tubes **Becton-Dickinson Catalog #352054**

Instrumentation required:

- a.Magnet (DynaMag™)
- b.Sample Mixer with rotation
- c.Laminar flow hood



Before start

If you need to obtain **CD4+ T cell for subsequent cell culture**, make sure you are using **sterile buffers** and **sterile plastic disposables** as well. Moreover, **work under laminar flow hood when you are processing samples** (from the beginning to the end of the following procedure). Otherwise, use non-sterile Buffers and disposables, and process samples in a cell isolation laboratory.

IMPORTANT NOTE: the isolation protocol is calibrated for using **25µL of beads for 10×10^6 PBMCs resuspended in 1mL**. For lower or higher cell number than 10×10^6 , resize the volumes, accordingly. (See also Table 1 on the data sheet of the kit).

ALL REAGENTS MUST BE AT ROOM TEMPERATURE WHEN USED!!!




- 1 Isolate PBMCs according either to the standard protocol from fresh blood or from buffy coat (PBMC- 01a - Isolation of Human PBMC from Buffy Coat, PBMC- 01b - Isolation of Human PBMC from Whole Blood).
- 2 Count the cells with Cellometer machine or by manual count, using either Trypan Blue or Türk solutions accordingly.

For automatic cell count with Cellometer machine use Trypan Blue.

Follow protocol **CELL COUNT- 03**

Document

 NAME
SOLUTION- 09 - Trypan Blue solution


CREATED BY
Farmacologia Medica

PREVIEW

For manual cell count use Türk solution for checking purity.

Follow protocol **CELL COUNT- 02**

Document

 NAME
SOLUTION- 08 - Türk solution

CREATED BY
Farmacologia Medica

PREVIEW



Equipment

Cellometer Auto T4

NAME

Automated cell counter


TYPE

Nexcelom Bioscience

BRAND

EuroClone

SKU

- 3 Resuspend Dynabeads in the vial using a vortex for >30 sec.
- 4 Transfer the desired volume of Dynabeads to a 5mL-tube (use BD tubes cat. n. 352054) following this proportion: **25µL of beads for 10×10⁶ cells.**
- 5 Add  2 µL of **Solution- 11** (found in the kit materials as Buffer 1), resuspend and place the tube into the magnet: beads will attach to the magnet very quickly (few seconds).

Discard then the supernatant by using a glass Pasteur pipette.

Remove the tube from the magnet.

Document




NAME

SOLUTION- 11 - CD4+T cells isolation buffer

CREATED BY


Farmacologia Medica

PREVIEW


- 6 Repeat the washing step 2 or 3 times to make sure that DMSO is all washed up.
- 7 After counting, centrifuge PBMCs sample at  1200 x g, 00:05:00 .

Equipment

Allegra AVANTI 30	NAME
Centrifuge	TYPE
Beckman Coulter	BRAND
Beckman Italy	SKU





- 8 Discard supernatant and resuspend pellet of 10×10^6 cells in  1 mL of **SOLUTION- 11**.

Document

	NAME
	SOLUTION- 11 - CD4+T cells isolation buffer
CREATED BY	
Farmacologia Medica	PREVIEW

- 9 Transfer cell suspension into the tube with beads, and resuspend vigorously.



- 10 Incubate the beads with cells for  00:20:00 at  4 °C with gentle rotation by putting the Sample Mixer in the fridge.
- 11 After incubation place the tube on the magnet and wait for 1-2 minutes, that is until the complex beads-cells is completely attached to the magnet.
- 12 While the tube is still in the magnet, carefully **remove** and **discard** the **supernatant** with a glass Pasteur pipette. 
- 13 Remove the tube from the magnet, add  2 µL of **SOLUTION- 11** and resuspend the cells very vigorously because of aggregates.

Document




NAME

SOLUTION- 11 - CD4+T cells isolation buffer

CREATED BY

Farmacologia Medica

PREVIEW

- 14 Repeat steps 11-13 twice (in total 3 times) to wash the bead-bound CD4+ T cells. These steps are critical to obtain a high purity of isolated cells.
- 15 Resuspend cell pellet in  100 µL of **SOLUTION- 07** (found in the kit materials as Buffer 2)
[The volume is calibrated for 10×10^6 cells, for lower or higher number of cell resize the volume accordingly].



Document





NAME

SOLUTION -07 - RPMI/FBS 1%

CREATED BY

Farmacologia Medica

PREVIEW

- 16 Add  10 μL of **DETACHaBEAD[®] CD4** for each 10×10^6 PBMCs.
(Resize this volume if the number of starting cell is different)
- 17 Add another  500 μL of **SOLUTION- 07** to increase the volume and transfer everything in a 1.5 mL eppendorf.

Document





NAME

SOLUTION -07 - RPMI/FBS 1%

CREATED BY


Farmacologia Medica

PREVIEW

- 18 Incubate  00:45:00 at  Room temperature (RT) with gentle rotation by using a Sample Mixer.
- 19 Transfer the sample from eppendorf to BD tube, and place the tube on magnet and wait for 1-2 mins, that is until the complex beads-cells is completely attached to the magnet.
- 20 While the tube is still in the magnet, **transfer the supernatant** containing the released cells into a 15 mL conical tube.





To obtain residual cells, wash the beads 3 times with  500 μ L of **SOLUTION- 07** and collect the supernatant each time.

Document





NAME

SOLUTION -07 - RPMI/FBS 1%

CREATED BY

Farmacologia Medica

PREVIEW

- 21 Add to the detached cell suspension **SOLUTION- 07** to a final volume of  5 mL and centrifuge at  1200 x g, Room temperature, 00:05:00

Document



NAME

SOLUTION -07 - RPMI/FBS 1%

CREATED BY

Farmacologia Medica

PREVIEW



Equipment

Allegra AVANTI 30

NAME

Centrifuge


TYPE

Beckman Coulter

BRAND

Beckman Italy

SKU

- 22 Resuspend the cells for cell counting in  1 mL of **SOLUTION- 07**: follow the appropriate protocol (see step 2 of this protocol).

Check the viability with Trypan blue.

Document



NAME

SOLUTION -07 - RPMI/FBS 1%

CREATED BY

Farmacologia Medica

PREVIEW

Document



NAME

SOLUTION- 09 - Trypan Blue solution

CREATED BY

Farmacologia Medica

PREVIEW

23 OPTIONAL STEP



Check the purity of the isolated CD4+ T cells by flow cytometry.

If needed, check the purity by labeling CD4 with the appropriate CD markers, such as CD3, CD4, CD8 and CD14 Ab and analyze samples with a flow cytometer to exclude the presence of undesired subsets.

Equipment

BD FACS Celesta

NAME

Flow Cytometer

TYPE

Becton Dickinson

BRAND

Milan Italy BD

SKU

24 EXPECTED RESULTS



Expected result

Cell Viability: $\geq 95\%$

Cell Yield: $\pm 4,6 \times 10^6$ cells starting from 25 mL of Fresh Blood
 $\pm 6 \times 10^6$ cells starting from 25 mL of Buffy Coat

If checked, purity of the isolated CD4+ cells must be $\geq 95\%$