BD Horizon™ Dri Treg Panel

5 Tests/kit

RESEARCH APPLICATION

The BD Horizon™ Dri Treg Panel tube can be used to identify different naïve and effector Treg subsets in human whole blood samples by multicolor flow cytometry.

PRODUCT DESCRIPTION

The BD Horizon Dri Treg Panel tube contains a dried, pre-aliquoted antibody cocktail comprising markers commonly used for the identification of regulatory T cell subsets. The dried multicolor panel has been performance-optimized in terms of fluorophore choice for each specificity as well as concentration of each conjugate.

KIT COMPONENTS

BD Horizon Dri Treg Panel contains the following:

- BD Horizon Dri Treg Panel

  The reagent is provided as 5 single-use tubes of dried reagent cocktail in a foil pouch.

- 1 compensation kit containing:
  - CD4 PerCP-Cy™5.5
  - CD45RA PE-Cy™7
  - CD127 APC-R700
  - CD3 APC-H7

  The compensation reagents are provided as one single-use tube of each conjugate in a dried format in a foil pouch. Only fluorochromes for tandem conjugates are included in this kit. Any other fluorochromes that need adjusting can be done using BD® FC Beads or other compensation beads.

Panel Composition

The panel comprises the following fluorochrome-conjugated antibodies:

Tube 1:

Specificity	Clone	Fluorochrome
CD25	2A3	BB515[a]
CD4	SK3	PerCP-Cy5.5
CD45RA	L48	PE-Cy7
CD161	DX12	APC
CD127	HIL-7R-M21	APC-R700
CD3	SK7	APC-H7

a. BD Horizon Brilliant™ Blue 515

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Tube 2:

Specificity	Clone	Fluorochrome
FoxP3	236A/E7	PE

The BD Horizon Dri Treg Panel can be used with additional liquid antibody drop-in reagents.

STORAGE AND HANDLING

Store tubes in their original foil pouch at 20°C-25°C. Do not freeze the reagent or expose it to direct light at any time during storage or incubation with blood samples. Once the pouch is opened, use the remaining tubes within 1 month when stored as directed. Do not use reagents beyond the expiration date indicated on the label.

CAUTION Due to the moisture sensitivity of the reagent, ensure that the pouch is immediately and completely resealed after removing a tube. Do not remove the desiccant from the reagent pouch.

WARNING

The reagents contain 6.4966% 1,2,3-propanetriol (CAS number 56-81-5), 0.7582% 2-methyl-4-isothiazolin-3-one (CAS number 2682-20-4), and 0.0977% sodium azide (CAS number 26628-22-8). The reagents are classified as hazardous according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS).

	Warning
	H317: May cause an allergic skin reaction. H412: Harmful to aquatic life with long lasting effects.
Prevention	P261: Avoid breathing dust/fume/gas/mist/vapors/spray. P272: Contaminated work clothing should not be allowed out of the workplace. P280: Wear protective gloves/protective clothing/eye protection/face protection. P271: Avoid release to the environment.
Response	P302+P352: IF ON SKIN: Wash with plenty of water. P333+P313: If skin irritation or rash occurs: Get medical advice/attention. P321: Specific treatment (see Safety Data Sheet). P363: Wash contaminated clothing before reuse.
Disposal	P501: Dispose of contents/container to an appropriate treatment and disposal facility in accordance with applicable laws and regulations, and product characteristics at time of disposal.

NOTE Source of all serum proteins is from USDA inspected abattoirs located in the United States.

PROCEDURE

Additional reagents and materials

- BD Pharmingen™ Transcription Factor Buffer Set (Catalog No. 562725)

  The buffer set is recommended for intracellular staining of Tregs. See the product technical data sheet (TDS) for the protocol.
- BD Pharmingen™ Stain Buffer (BSA) (Catalog No. 554657)
- If creating user-defined reference settings:
  - BD® FC Beads BB515 (Catalog No. 661631)
  - BD® FC Beads APC (Catalog No. 661620)
  - BD® FC Beads PE (Catalog No. 661616)

Preparing the sample

Use aseptic blood collected in EDTA blood collection tubes. Individual assay performance criteria should be evaluated as fixation and permeabilization buffers can alter epitopes and fluorochromes.

1. Isolate peripheral blood mononuclear cells (PBMCs) and resuspend at 1×10^7 cells/mL.
2. Remove a dried reagent Tube 1 from the reagent pouch and the 4 tubes from the compensation pouch, and place them in a rack, protected from light.

   The compensation tubes are stained the first time you run the assay.

   NOTE You can prepare compensation tubes for the other fluorochromes using BD FC Beads or other compensation beads, if needed.
3. Immediately reseal the pouch containing the remaining reagent tubes.
4. Add 100 µL (1×10^6) isolated PBMCs to the bottom of each tube.
5. Gently pipette/vortex the tube for 4 seconds to reconstitute the dried reagent.
6. Incubate 30 minutes at room temperature, protected from exposure to direct light.
7. Wash cells one time with BD Pharmingen Stain Buffer (BSA).

   For the compensation tubes, continue with step 15.
8. Add 1 mL of 1X TF Fix/Perm Buffer.
9. Incubate 50 minutes at 4°C, protected from exposure to direct light.
10. Wash cells twice with 1X TF Perm/Wash Buffer and resuspend cells in 100 µL of the same buffer.
11. Transfer cell suspension to the bottom of the dried reagent Tube 2.
12. Gently pipette/vortex the tube for 4 seconds to reconstitute the dried reagent.
13. Incubate 50 minutes at 4°C, protected from exposure to direct light.
14. Wash cells twice with 1X TF Perm/Wash Buffer.
15. Resuspend cells in 350 µL BD Pharmingen Stain Buffer (BSA).

    The sample is now ready for acquisition.

Acquiring the sample

To acquire the stained sample on a BD FACSLyric™ flow cytometer:

1. Vortex the tube briefly and install it on the instrument.
2. Preview the sample to determine whether the photomultiplier tube voltages (PMTVs) and/or the compensation values need to be adjusted.
   a. If no adjustments are needed, keep the default Lyse/Wash tube settings for acquisition.
   b. If the PMTVs are adjusted, create tube settings for the new PMTVs.
   c. If compensation values need to be adjusted, create user-defined reference settings by acquiring the stained compensation tubes, and create tube settings.

      See the *BD FACSLyric™ Reference System* for more information.
3. Acquire the sample using the optimal tube settings.
4. Analyze the sample.

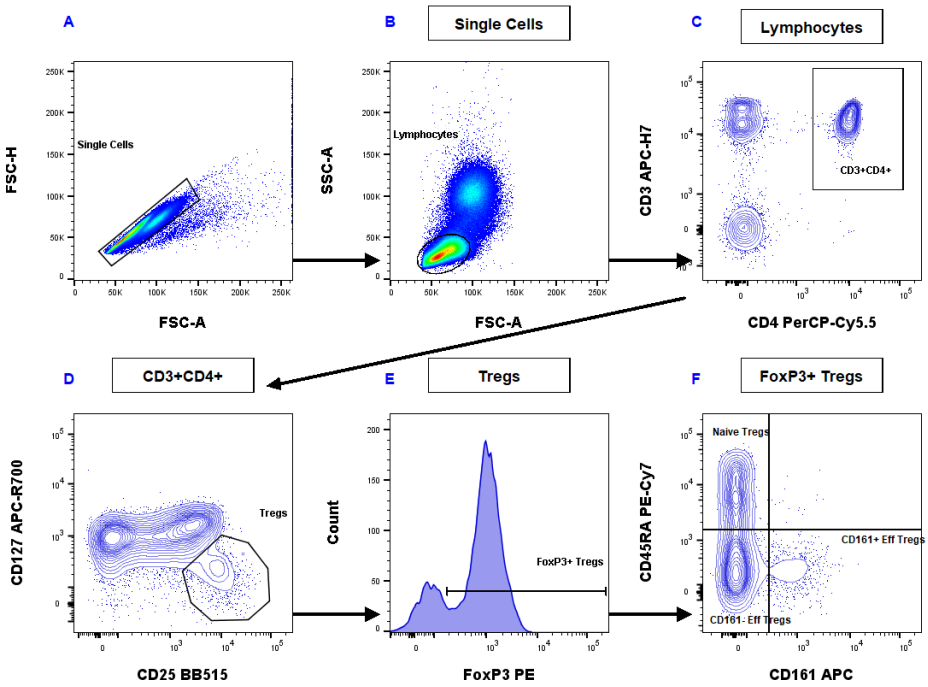
NOTE A BD FACSuite™ template for acquisition is available from your local BD sales representative.

NOTE For sample acquisition on other flow cytometers, please follow the manufacturer's instructions for compensation setup.

Analyzing the data

Flow cytometric analysis was performed on freshly isolated normal PBMCs stained using the BD Horizon Dri Treg Panel. Stained samples were acquired using a BD FACSLyric flow cytometer.

Figure 1 Representative data analysis using the BD Horizon Dri Treg Panel



To analyze the Treg cells:

1. Create an FSC-A vs FSC-H dot plot and draw a gate around the singlet population (A).
2. Create an FSC-A vs SSC-A dot plot and apply the singlet gate. Draw a gate around the lymphocyte population (B).
3. Create a CD4 PerCP-Cy5.5 vs CD3 APC-H7 dot plot and apply the lymphocyte gate. Draw a gate to encompass the $CD3^+CD4^+$ lymphocytes (C).
4. Create a CD25 BB515 vs. CD127 APC-R700 dot plot and apply the $CD3^+CD4^+$ gate. Draw a gate to encompass the Treg cells ($CD25^{bright}/CD127^{dim}$ (D).
5. Create a histogram of FoxP3 PE and apply the Tregs gate. Draw a gate to include only $FoxP3^+$ Treg peak (E).
6. Create a CD161 APC vs CD45RA PE-Cy7 dot plot and apply the $FoxP3^+$ Tregs gate. Create a quadrant gate to identify Naïve and Effector Treg cells (F).

WARRANTY

Unless otherwise indicated in any applicable BD general conditions of sale for non-US customers, the following warranty applies to the purchase of these products.

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REFERENCES

1. 1.Miyara M, Chader D, Sage E, Sugiyama D, Nishikawa H, Bouvry D, Claër L, Hingorani R, Balderas R, Rohrer J, Warner N, Chapelier A, Valeyre D, Kannagi R, Sakaguchi S, Amoura Z, Gorochov G. Sialyl

Lewis (CD15s) identifies highly differentiated and most suppressive FOXP3high regulatory T cells in humans. *PNAS*. 2015 Jun 9;112(23):7225-7230.

2. Pesenacker AM, Bending D, Ursu S, Wu Q, Nistala K, Wedderburn LR. CD161 defines the subset of FoxP3+ T cells capable of producing proinflammatory cytokines. *Blood*. 2013 Apr 4;121(14):2647-2658.

3. Afzali B, Mitchell PJ, Edozie FC, Povoleri GA, Dowson SE, Demandt L, Walter G, Canavan JB, Scotta C, Menon B, Chana PS, Khamri W, Kordasti SY, Heck S, Grimbacher B, Tree T, Cope AP, Taams LS, Lechler RI, John S, Lombardi G. CD161 expression characterizes a subpopulation of human regulatory T cells that produces IL-17 in a STAT3-dependent manner. *Eur J Immunol*. 2013 Aug;43(8):2043-2054.

PATENTS AND TRADEMARKS