BD Horizon™ Dri Memory T Cell Panel

5 Tests/kit

RESEARCH APPLICATION

The BD Horizon™ Dri Memory T Cell Panel tube can be used to identify different naïve and memory T cell subsets in human whole blood by multicolor flow cytometry.

PRODUCT DESCRIPTION

The BD Horizon Dri Memory T Cell Panel tube contains a dried, pre-aliquoted antibody cocktail comprising markers commonly used for the identification of naïve and memory T cells. The dried multicolor panel has been performance-optimized in terms of fluorochrome choice for each specificity as well as concentration of each conjugate. It is designed for use on a flow cytometer that has blue, red, and violet lasers.

KIT COMPONENTS

BD Horizon Dri Memory T Cell Panel contains the following:

- BD Horizon Dri Memory T Cell Panel

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

- 1 compensation kit containing:
  - CD95 PE-Cy™7
  - CD27 APC-R700
  - CD3 APC-H7
  - CD45RA BV605

  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:

Specificity	Clone	Fluorochrome
CD197	150503	FITC
CD95	DX2	PE-Cy7
CD27	M-T271	APC-R700
CD3	SK7	APC-H7
CD4	SK3	V450[a]
CD8	SK1	V500-C[a]
CD45RA	HI100	BV605[a]

a. BD Horizon™ V450, BD Horizon™ V500-C, BD Horizon Brilliant™ Violet 605

For Research Use Only. Not for use in diagnostic or therapeutic procedures.

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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 Horizon™ Brilliant Stain Buffer (Catalog No. 659611)
- BD FACS™ Lysing Solution (Catalog No. 349202)
- BD Pharmingen™ Stain Buffer (BSA) (Catalog No. 554657)
- If creating user-defined reference settings:
  - BD® FC Beads FITC (Catalog No. 661615)
  - BD® FC Beads V450 (Catalog No. 661623)
  - BD® FC Beads V500-C (Catalog No. 661624)

Staining the sample

Use aseptic blood collected in EDTA blood collection tubes.

1. Remove a tube from the reagent pouch and the 5 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.

2. Immediately reseal the pouch containing the remaining reagent tubes.

3. Add 50 µl of BD Horizon™ Brilliant Stain Buffer into the bottom of the reagent tube.
4. Gently pipette/vortex the tube for 4 seconds to reconstitute the dried reagent.
5. Add 100 µl of well-mixed anti-coagulated whole blood or bone marrow to each of the tubes and vortex the tube for 4 seconds.
6. Incubate 30 minutes at room temperature, protected from exposure to direct light.
7. Add 2 ml of 1X BD FACS Lysing Solution to the tube. Vortex to mix.
8. Incubate for 10 minutes at room temperature, protected from light.
9. Centrifuge at 540*g* for 5 minutes at room temperature.
10. Aspirate the supernatant and resuspend cells with BD Pharmingen Stain Buffer (BSA).
11. Repeat steps 9-10 one additional time.
12. Centrifuge at 540*g* for 5 minutes at room temperature.
13. Aspirate the supernatant and gently vortex.
14. Resuspend the cell pellet in 500 µl of the stain buffer.

    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 appropriate 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.

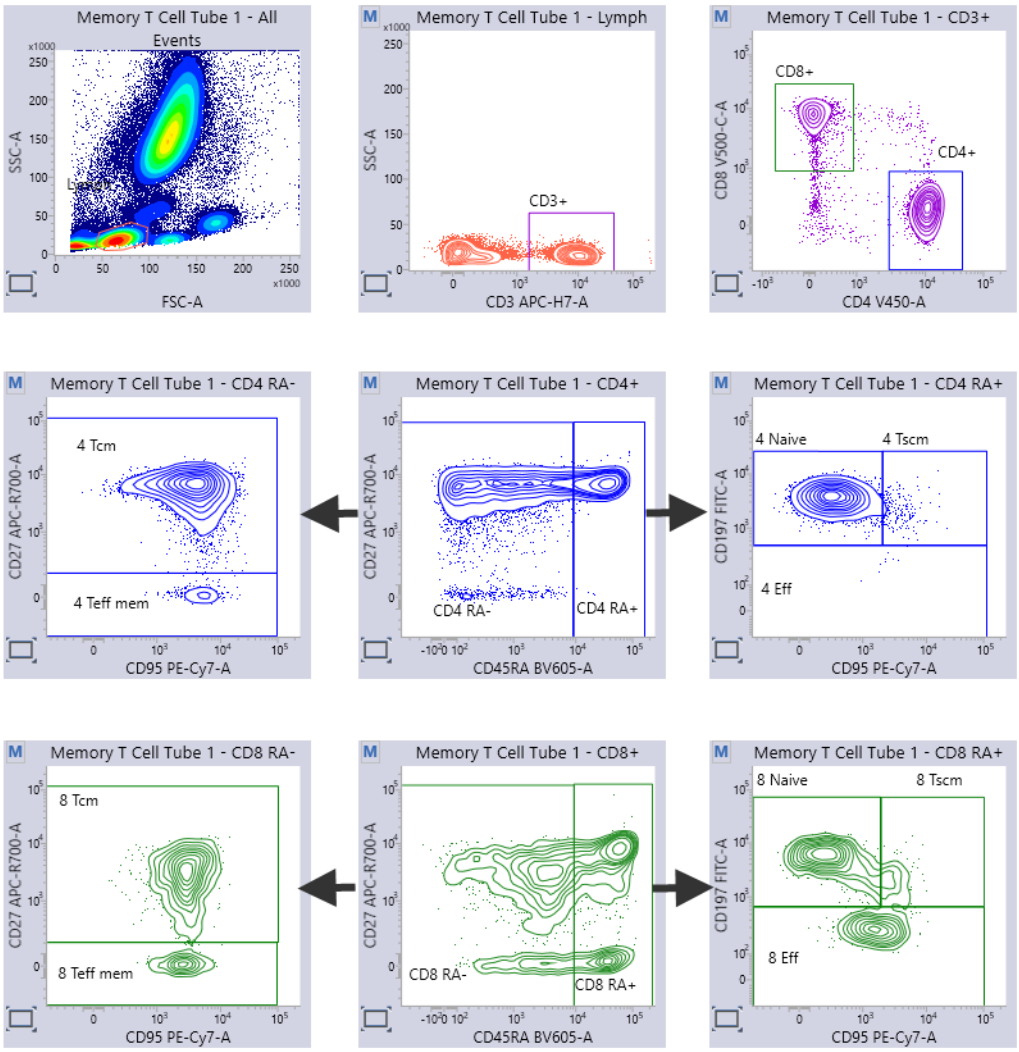
NOTE A BD FACSuite™ template for acquisition is available from your local BD sales representative.

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 normal fresh blood stained using the BD Horizon Dri Memory T Cell Panel. Stained samples were acquired on a BD FACSLyric flow cytometer.

Figure 1 Example data analysis for BD Horizon Dri Memory T Cell Panel



To analyze the data:

1. Create an FSC-A vs SSC-A dot plot and draw a gate around the lymphocyte population.
2. Create a CD3 APC-H7-A vs SSC-A dot plot with the lymphocyte gate applied. Draw a gate to encompass $CD3^+$ events.
3. Create a CD4 V450-A vs CD8 V500-C-A dot plot with the $CD3^+$ gate applied. Create gates to encompass single-positive $CD4^+$ and $CD8^+$ events.
4. Create a CD45RA BV605-A vs CD27 APC-R700-A dot plot and apply the $CD4^+$ gate. Create gates to encompass the $CD45RA^-$ and $CD45RA^+$ subpopulations.
5. Create a CD95 PE-Cy7-A vs CD27 APC-R700-A dot plot and apply the $CD45RA^-$ gate. Create gates to encompass $CD27^+$ cells (central memory cells [Tcm]) and $CD27^-$ cells (effector memory cells [Teff mem]).
6. Create a CD95 PE-Cy7-A vs CD197 FITC-A dot plot and apply the $CD45RA^+$ gate. Create gates to encompass $CD197^+CD95^-$ cells (naïve cells), $CD197^+CD95^+$ cells (stem memory cells [Tscm]), and $CD197^-$ cells [effector cells [Eff]).
7. Create dot plots to repeat steps 4–6, but apply the $CD8^+$ gate in the first step, to identify the $CD8^+$ memory subpopulations.

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

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3. Sallusto F, Geginat J, Lanzavecchia A. Central memory and effector memory T cell subsets: function, generation, and maintenance. *Annual Review of Immunology*. 2004; 22:745–763.

PATENTS AND TRADEMARKS

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