



BD Horizon™ Dri Monoset Panel

5 Tests/kit

RESEARCH APPLICATION

The BD Horizon™ Dri Monoset Panel can be used to identify monocyte subsets – classical (M1), intermediate (M2), and non-classical (M3) – in human whole blood samples by multicolor flow cytometry. Monocyte subset percentages can be measured directly with the panel. When used with BD Trucount™ Tubes, absolute counts of each monocyte subset can be enumerated. Median expression levels of HLA-DR on each monocyte subset can be calculated in antibody bound per cell (ABC) units after calibration of median fluorescence intensity (MFI) in the PE channel using BD Quantibrite™ Beads.

PRODUCT DESCRIPTION

The BD Horizon Dri Monoset Panel tube contains a dried pre-aliquoted antibody cocktail containing commonly used markers for the identification and characterization of monocyte subsets. The formats and concentration for each specificity have been optimized to work as a combined panel.

KIT COMPONENTS

The BD Horizon Dri Monoset Panel is provided as 5 single-use tubes of dried reagent cocktail in a foil pouch.

Panel Composition

The panel comprises the following fluorochrome-conjugated antibodies:

Specificity	Clone	Fluorochrome
CD16	3G8	FITC
HLA-DR	L243	PE
CD14	MφP9	PerCP
CD192 (CCR2)	LS132.1D9	APC

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 cells. 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 the pouch is immediately and completely resealed after removing a tube. Do not remove the desiccant from the reagent pouch.


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

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WARNING

The reagent contains 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 reagent is 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 FACSTTM Lysing Solution (Catalog No. 349202)
- Phosphate buffered saline (PBS)
- BD Trucount Tubes, if calculating absolute cell counts (Catalog No. 340334)
- BD Quantibrite Beads, if calculating ABD units (Catalog No. 340495)
- If creating user-defined reference settings:
 - BD® FC Beads FITC (Catalog No. 661615)
 - BD® FC Beads PE (Catalog No. 661616)
 - BD® FC Beads PerCP (Catalog No. 661618)
 - BD® FC Beads APC (Catalog No. 661620)

Staining the sample

Use whole blood collected in EDTA blood collection tubes.

1. Add 50 µl of well-mixed, fresh EDTA-anticoagulated whole blood into the bottom of the dried reagent cocktail tube.
2. Gently pipette/vortex tube for 4 seconds to completely reconstitute the dried antibodies.
3. Incubate 30 minutes at room temperature, protected from exposure to direct light.
4. Add 450 µl of 1X BD FACS Lysing Solution to the tube. Vortex to mix.
5. Incubate for 30 minutes at room temperature, protected from light.
6. Add 1.5 mL of PBS and vortex to mix.

The sample is ready to be acquired on a flow cytometer with appropriate compensation settings for FITC, APC, PE and PerCP.

Acquiring the sample

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

1. Confirm that the tube of stained cells is ready to be acquired.
See the following sections, describing preparation of the stained sample for acquisition, depending on whether you are determining monocyte subset percentages, calculating absolute counts, or measuring median expression levels of HLA-DR.
2. Vortex the tube briefly and install it on the instrument.
3. 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/No-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.
4. Acquire the sample using the optimal tube settings.
5. Analyze the sample.

If determining monocyte subset percentages:

1. (Optional): To reduce the time needed to acquire the sample,
 - a. Centrifuge the tube at 500g for 5 minutes.
 - b. Remove 1.5 mL of the supernatant.
2. Vortex tube thoroughly to resuspend the stained cells.
3. Acquire the sample.

If calculating absolute counts of monocyte subsets:

1. Transfer 1.5 mL of the stained cell suspension to a BD Trucount Tube.
2. Vortex to mix.
3. Acquire the sample.

If the median expression levels of HLA-DR on monocyte subsets are to be measured (in ABC units):

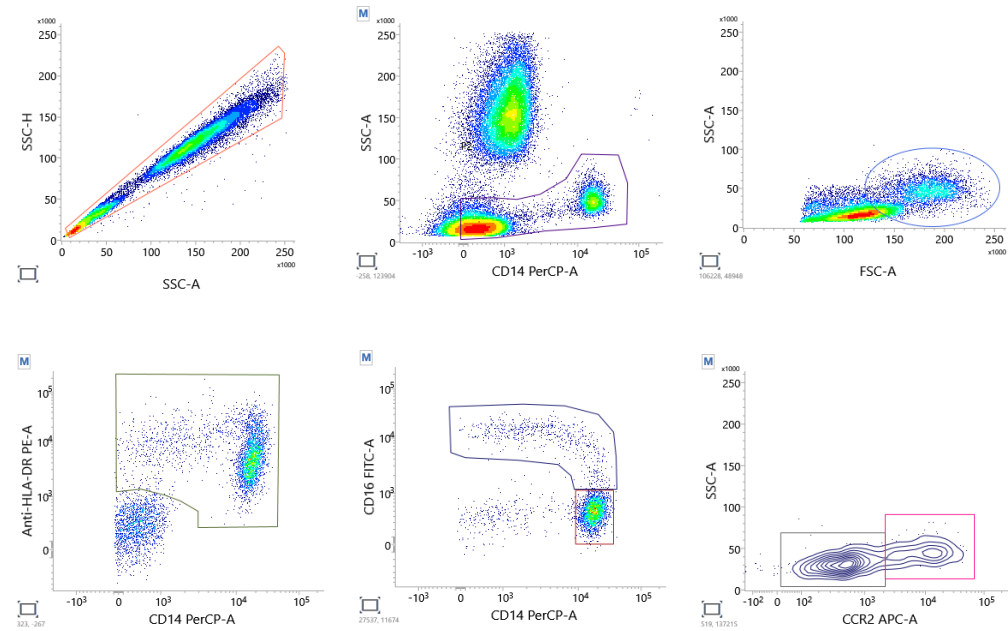
1. Prepare a tube of BD Quantibrite Beads according to the product data sheet.
2. Acquire the beads on the flow cytometer.
3. Vortex the tube of stained cells.
4. Acquire the stained cells.

NOTE Acquire the beads and the stained cells using the same PE channel PMTV.

5. Calculate the ABC units of the HLA-DR marker on each monocyte subset.
Follow the instructions for manual analysis found in the *BD Quantibrite™ Beads* data sheet.

The monocyte subset gating strategy is based on recommendations by the European Society of Cardiology Working group¹ with the addition of an HLA-DR gate to improve the identification of monocytes. An example of gating for monocyte subsets using data acquired on a BD FACSLyric™ flow cytometer and analyzed using BD FACSuite™ software is shown in Figure 1.

Figure 1 Monocyte subset analysis with the BD Horizon Dri Monoset Panel.



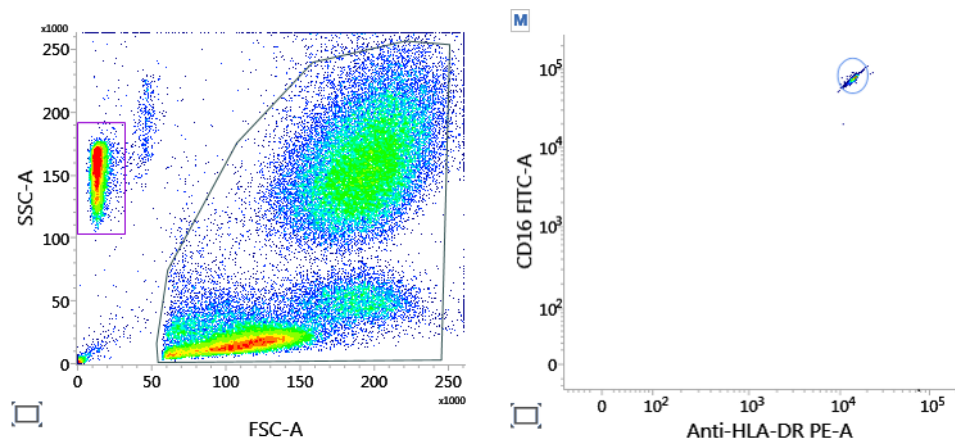
To analyze the monocyte subsets:

1. Create an SSC-A vs SSC-H dot plot and draw a gate around the singlet population.
2. Create a CD14 PerCP-A vs SSC-A dot plot and apply the SSC singlet gate. Draw a gate to encompass the SSC^{lo} populations (CD14⁺ and CD14^{+/-}).
3. Create an FSC-A vs SSC-A dot plot and apply the CD14 gate. Draw a gate around the population with high forward scatter.
4. Create a CD14 PerCP-A vs HLA-DR PE-A dot plot and apply the FSC gate. Draw a gate to encompass HLA-DR⁺ cells.
5. Create a CD14 PerCP-A vs CD16 FITC-A dot plot and apply the HLA-DR gate. Draw two gates, the first gate encompassing CD14⁺CD16^{lo} cells (classical [M1] monocytes), and the second gate encompassing CD16⁺ cells.
6. Create a CCR2 APC-A vs SSC-A dot plot and apply the CD16⁺ gate. Draw two gates, the first gate encompassing CCR2^{hi} cells (intermediate [M2] monocytes), and the second gate encompassing CCR2^{lo} cells (non-classical [M3] monocytes).
7. Calculate relative percentages of monocyte subsets from the cell counts.

To identify the Trucount beads population:

1. Create an FSC-A vs SSC-A and draw two gates, the first gate encompassing the BD Trucount beads population, and the second gate encompassing the cell population.

Figure 2 BD Trucount beads gating strategy



NOTE The threshold setting might have to be changed to include the smaller-sized Trucount beads.

2. Create an HLA-DR PE-A vs CD16 FITC-A dot plot and apply the Trucount beads gate. Draw a gate to encompass the beads population.
3. Analyze the cell population, identified in the first dot plot, using the gating strategy described previously.
4. Calculate the absolute count of the desired population using the following equation:

$$\frac{\# \text{ events in cell population}}{\# \text{ events in Trucount beads region}} \times \frac{\# \text{ beads/test}}{\text{test volume } (\mu\text{L})} \times 40 = \text{cell population absolute count (cells}/\mu\text{L})$$

The test volume is 1500 μL .

The # beads/test is found on the BD Trucount Tubes pouch label and varies from lot-to-lot.

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.

THE PRODUCTS SOLD HEREUNDER ARE WARRANTED ONLY TO CONFORM TO THE QUANTITY AND CONTENTS STATED ON THE LABEL OR IN THE PRODUCT LABELING AT THE TIME OF DELIVERY TO THE CUSTOMER. BD DISCLAIMS HEREBY ALL OTHER WARRANTIES, EXPRESSED OR IMPLIED, INCLUDING WARRANTIES OF MERCHANTABILITY AND FITNESS FOR ANY PARTICULAR PURPOSE AND NONINFRINGEMENT. BD'S SOLE LIABILITY IS LIMITED TO EITHER REPLACEMENT OF THE PRODUCTS OR REFUND OF THE PURCHASE PRICE. BD IS NOT LIABLE FOR PROPERTY DAMAGE OR ANY INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING PERSONAL INJURY, OR ECONOMIC LOSS, CAUSED BY THE PRODUCT.

REFERENCES

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TRADEMARKS

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