

# DryFlowEx ASC Screen kit

Cat. No. ED7704

Specificity	IgD	CD27	CD24	CD19	CD21	CD38	CD45
Clone	IA6-2	LT27	SN3	LT19	LT21	HIT2	2D1
Isotype (murine)	IgG2a	IgG2a	IgG1	IgG1	IgG1	IgG1	IgG1
Fluorochrome	FITC	PE	PerCP-Cy™5.5	PE-Cy™7	APC	APC-Cy™7	Pacific Blue™
λ excitation	488 nm	488 nm	488 nm	488 nm	633 nm	633 nm	405 nm
Emission maxima	525 nm	575 nm	692 nm	780 nm	660 nm	780 nm	455 nm

## Description

DryFlowEx ASC Screen Kit is designed for Flow Cytometry screening of Antibody Secreting B Cells (ASC) during viral infection.

Multicolor panel of antibody conjugates CD45/IgD/CD27/CD24/CD19/CD21/CD38 dried in a single flow cytometry tube enables immunophenotyping of differentiated B lymphocyte subsets in EDTA-anticoagulated human blood.

## Reagents provided

ASC Screen 5T Pack (ED7704-5T) containing 5 pcs of single-test tubes for flow cytometry (12x75 mm).

Cat. No.	Product	Amount
ED7704-5T	ASC Screen 5T Pack	10 packs

ASC Screen Compensation Tubes Pack (ED7704-CT) containing 7 pcs of single-test compensation tubes for flow cytometry (12x75 mm).

Cat. No.	Product	Amount
ED7704-CT	ASC Screen Compensation Tubes Pack	1 pack
ED7704-CT1	CD45 Pacific Blue™	1 pc
ED7704-CT2	IgD FITC	1 pc
ED7704-CT3	CD27 PE	1 pc
ED7704-CT4	CD24 PerCP-Cy™5.5	1 pc
ED7704-CT5	CD19 PE-Cy™7	1 pc
ED7704-CT6	CD21 APC	1 pc
ED7704-CT7	CD38 APC-Cy™7	1 pc

## Materials required but not provided

Ultrapur, deionized water  
Pipettes and dispensable pipette tips  
Lysing solution EXCELLYSE Easy (cat. No. ED7066)

## Storage and handling

The product ED7704 retains its functional characteristics when stored at laboratory temperature until the specified expiry date printed on product label.

Use immediately after opening. Do not reuse. Do not expose to direct sunlight. Do not freeze the product.

Do not use the product after the expiry date printed on the product label.

ALU pouches of ASC Screen 5T Pack (ED7704-5T) containing 5 test tubes must be opened directly before use. Reseal unused ED7704-5T test tubes in the ALU pouch containing drying silica gel using the pouch zip-lock.

Once the ALU pouch ED7704-5T is opened, use the remaining single-test tubes within the period of one month.

## Warnings and precautions

Intended for research use only. Blood samples are considered as potentially infectious and must be handled with care. Use protective gloves and follow procedures for handling potentially infectious materials. Avoid contact of human blood samples with skin, eyes and mucous membranes. It is highly advised to close test tube using tube caps to minimize risk of exposure to infectious specimen.

Do not use specimen with signs of hemolysis. A flow cytometer equipped with violet (405 nm), blue (488 nm) and a red (640 nm) laser with appropriate fluorescence detectors must be used for sample analysis. The flow cytometer must be maintained and calibrated on a regular basis for stable fluorescence detector sensitivity.

Improperly compensated multiparameter flow cytometry data may be interpreted incorrectly. A set of compensation tubes (Cat. No. ED7704-CT) is a part of the product. These tubes contain the same dried single-color antibodies, as the dried test tube and provide positive and negative cell population when used for staining. Compensation tubes must be handled the same way as Test tubes.

Any application non-compliance may affect test results.

## Application

### Specimen

Use peripheral whole blood collected into a sterile tube containing anticoagulant (EDTA). Store the collection tube at room temperature prior to staining. The specimen must be stained within 48 hours after collection.

### Lysing solution preparation

Prepare freshly diluted 1x EXCELLYSE Easy (Cat. No. ED7066). Lysing solution is provided as a 10x concentrate and must be diluted 10x using deionized water prior to use (1 part of reagent into 9 parts of water). Prepare 2ml of diluted lysing solution per 1 tube. Assign with the date of preparation and store at 2 - 25 °C for maximum of 4 weeks.

### Test tube and compensation tube preparation

- Add 100 µl of peripheral whole blood to the Test tube or the Compensation tube. Blood specimen does not need to be washed prior to staining. Concentration of IgD antibodies present in the specimen is not sufficient to affect test results.
- Vortex excessively for a minimum of 7 seconds. Note: Shortening the vortex time may negatively affect test results.
- Incubate the tube for 20 minutes at room temperature in the dark.
- Lyse the erythrocytes by adding 2 ml of 1x EXCELLYSE Easy to the tube.
- Incubate the tube for 10 minutes at room temperature in the dark.
- Centrifuge the tube for 5 minutes at 300xg.
- Discard supernatant and resuspend the cell pellet using 0,2-0,5 ml PBS.
- Analyze the sample within 2 hours after staining.

### Cytometer set-up

Set the flow cytometer fluorescence detector voltages to Euroflow® protocol using Rainbow calibration particles, 8 peaks (Spherotech Inc., Cat. No. RCP-30-5A (Euroflow)). BD FACS cytometers may also be set using BD™ CS&T Beads. Beckman Coulter cytometers may also be set using Flow-Set Pro Fluorospheres.

- van Dongen JJ et al., 2012, Leukemia
- <https://www.spherotech.com/Calibration-Particles.htm>

Set „Protocol“ for Beckman Coulter flow cytometers and „Experiment“ for BD flow cytometers to acquire data from detectors:

- Forward Scatter (signal height)
- Forward Scatter (signal area)
- Side Scatter (signal area)
- FITC (FL1 for Beckman Coulter Navios®)
- PE (FL2 for Beckman Coulter Navios®)
- PerCP-Cy™5.5 (FL4 for Beckman Coulter Navios®)
- PE-Cy™7 (FL5 for Beckman Coulter Navios®)
- APC (FL6 for Beckman Coulter Navios®)
- APC-Cy™7 (FL8 for Beckman Coulter Navios®)
- Pacific Blue™ (FL9 for Beckman Coulter Navios®)

Prepare a compensation matrix to eliminate fluorescence spillover or use compensation matrix prepared from the same lot of compensation tubes ED7704-CT. Follow your flow cytometer manufacturers instructions. Compensation tubes must be prepared the same way as test tubes (See Test tube and Compensation tube preparation).

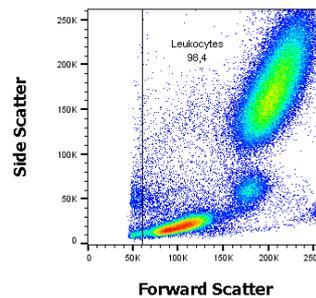
After setting a compensation matrix, measuring Compensation tubes and selecting positive and negative cell events BD FACSDiva™ software generates compensation matrix automatically.

In Navios software it is required to compensate spillover between required fluorescence detectors manually using „sliders“ in compensation mode.

## Flow Cytometry Analysis

1. Set threshold („Discriminator“ for Navios® cytometers) on event size (Fig. 1), or Fluorescence Intensity in Pacific Blue™ detector (FL9), or both, so that minimum 90 % of all recorded events are leukocytes. Very high Threshold / Discriminator value settings may cause irreversible loss of lymphocyte events from analysis.

Figure 1. Depiction of sufficient event size Threshold settings in a Forward vs. Side Scatter dot-plot



2. Acquire minimum of 200 000 events. Due to overall low percentage of target cell population it is of most importance to acquire the highest amount of leukocyte events possible.

### Data analysis and phenotyping procedure

Analyze measured data in FlowJo software, BD FACSDiva™ or Navios EX acquisition SW. Check for proper fluorescence spillover compensation. Non-compensated data may be compensated post data acquisition after applying pre-calculated or pre-saved compensation matrix.

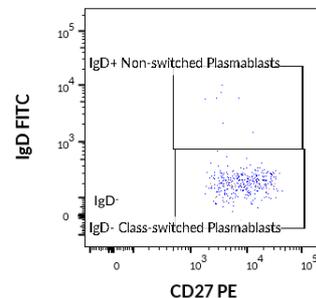
Identify ASC subsets (target cell populations) according to Phenotyping strategy, see Appendix No. 1.

### Data interpretation

Percentage of ASC subsets from all CD19+ B lymphocytes (Fig. 2) may change during a viral infection.

An increase in ASC subset percentage may be detected with the viral infection onset. This percentage is expected to change further as the viral infection proceeds.

Figure 2. Flow cytometry dot-plot depiction of target ASC subsets: IgD Class-switched plasmablasts and IgD non-switched Plasmablasts in normal human peripheral whole blood labeled using ED7704-5T.



## Literature

This product and its use have not been published yet.

## Manufacturer

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## Trademarks

- BD FACS, BD™ CS&T Beads, BD FACSDiva™ and FlowJo® are registered trademarks of Becton Dickinson
- Navios® is a registered trademark of Beckman Coulter

## Revision history

- Version 5

## Symbols

- REF** Catalog number
- LOT** Batch code
- Use-by date
- Temperature limits
- Consult instructions for use
- Keep away from sunlight
- Manufacturer
- RUO** For Research use only.  
Not for use in diagnostic or therapeutic procedures.



## DryFlowEx ASC Screen kit

50×1 test | Cat. No. ED7704

**For Research use only.**

**Not for use in diagnostic or therapeutic procedures.**

Technical Data Sheet

Version ED7704\_TDS\_v5\_EN

Date of Issue: 21.04.2020



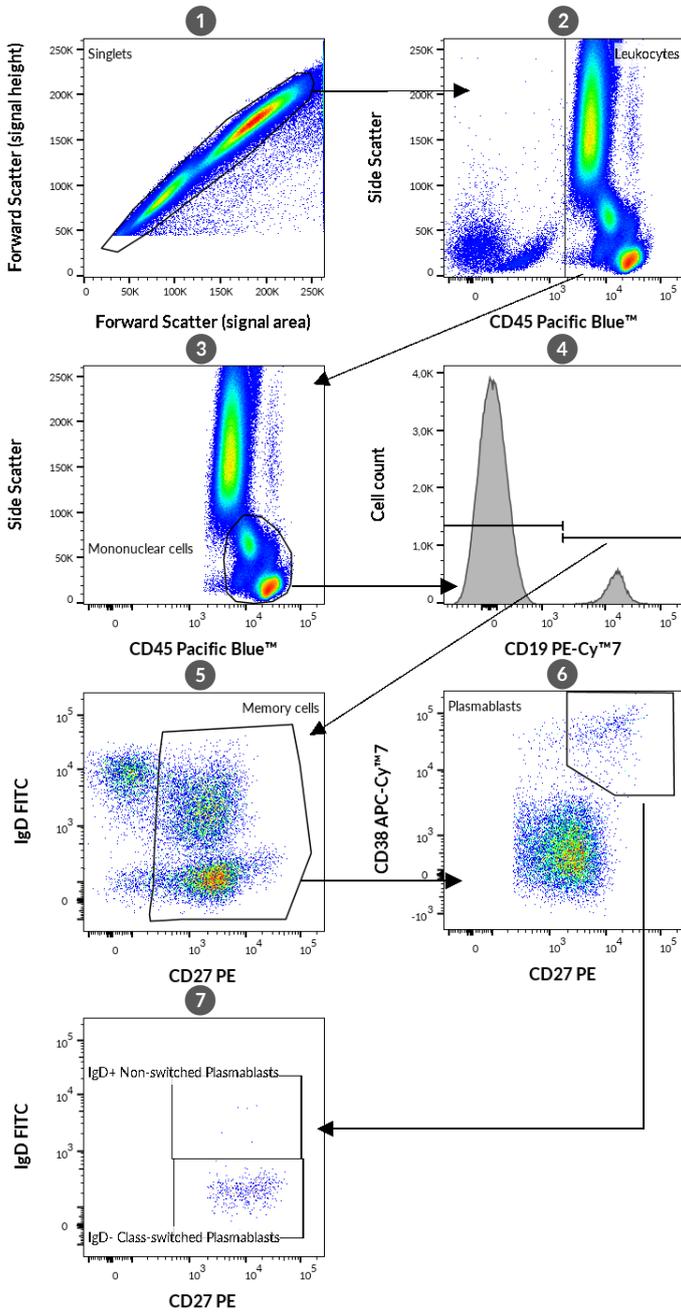
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**Appendix No. 1 - Phenotyping strategy**



- 1 Singlet events
- 2 CD45<sup>+</sup> singlets - leukocytes
- 3 CD45<sup>+</sup> SSC<sup>low</sup> mononuclear cells
- 4 CD45<sup>+</sup>CD19<sup>+</sup> lymphocytes (B lymphocytes)
- 5 CD45<sup>+</sup>CD19<sup>+</sup>CD27<sup>+</sup> B lymphocytes (memory B cells)
- 6 CD45<sup>+</sup>CD19<sup>+</sup>CD27<sup>+</sup>CD38<sup>+</sup> Plasmablasts
- 7 ASC target populations:
  - IgD<sup>+</sup> Non-switched Plasmablasts
  - IgD<sup>-</sup> Class-switched Plasmablasts

More B lymphocyte subsets to be evaluated during viral infection:

Transitional B cells

- CD45<sup>+</sup> CD19<sup>+</sup> CD27<sup>-</sup> CD24<sup>++</sup> CD38<sup>+</sup> (CD21<sup>+</sup> IgD<sup>+</sup>)
- Do not secrete antibodies

Marginal zone-like B cells

- CD45<sup>+</sup> CD19<sup>+</sup> CD27<sup>+</sup> IgD<sup>+</sup> (CD21<sup>+/++</sup> CD24<sup>+</sup> CD38dim)
- T cell independent immune response

CD21<sup>low</sup> B cells

- CD45<sup>+</sup> CD19<sup>+</sup> CD21<sup>-</sup> CD38<sup>-</sup>
- Auto-immunity connected cells

Näive B cells

- CD45<sup>+</sup> CD19<sup>+</sup> CD27<sup>-</sup> CD24<sup>+</sup> CD38<sup>-</sup> (IgD<sup>+</sup>)