BD OneFlow™ Setup Beads

25 tests per kit—Catalog No. 658620

23-15758(02)
2023-07
English

CE IVD

1. INTENDED USE

BD OneFlow™ Setup Beads are intended to set voltages appropriate for the BD multicolor tube assay when used with a suitably equipped BD flow cytometer and software designated for in vitro diagnostic use.

2. SUMMARY OF THE TEST

BD OneFlow™ Setup Beads are fluorescent particles that are used to set cytometer detector photomultiplier tube voltages (PMTVs) for the BD multicolor tube assay. PMTVs are manually adjusted to place the BD OneFlow™ Setup Beads at their lot specific median fluorescence intensity (MFI) target ranges for all fluorescence parameters. Lysed washed blood (LWB) is used to set cytometer FSC and SSC voltages to a target value range. The detector settings are then saved as Application Settings.

BD OneFlow™ Setup Beads are intended for use by laboratory professionals.

Principle of Operation

BD has developed a suite of beads that are used with BD FACSDiva™ software to standardize setup of the BD FACSCanto™ II flow cytometer with a 3-laser, 8-color 4-2H-2V BD default (4-2H-2V) optical configuration. First, BD FACSDiva™ CS&T IVD Beads are used to perform daily cytometer quality control. BD OneFlow™ Setup Beads and LWB are then used to set assay-specific PMTVs and to generate Application Settings. Finally, BD® FC Beads 8-Color Kit for BD OneFlow™ Assays (BD® FC Beads) is used to calculate compensation.

3. REAGENT

Reagent Composition

BD OneFlow™ Setup Beads are supplied in phosphate buffered saline (PBS) with bovine serum albumin (BSA) and 0.1% sodium azide.

Precautions

- Do not use BD OneFlow™ Setup Beads beyond their expiration date or beyond the day-of-use stability period after dilution, as described in the Storage and Handling section. Beads used beyond their stability period begin to lose fluorescence, which may result in inaccurate PMTV setup.
- MFI target ranges provided on the monthly MFI target range card are bead lot specific. Verify that the bead lot number on the monthly MFI target range card matches the lot ID of the BD OneFlow™ Setup Beads that you are using. A mismatch will result in inaccurate PMTVs and Application Settings.
- Go to regdocs.bd.com/regdocs/sdsSearch to download the Safety Data Sheet.

Storage and Handling

- Store the vial at 2–8 °C. The vial should not be frozen. Protect from exposure to light. The beads are stable until the expiration date shown on the vial label when stored as directed. Do not use after the expiration date. Do not mix the contents of one kit with another. Target values can vary between lots and this could result in inaccurate detector settings.
- After dilution, the beads are stable for
    - 1 hour at 18–25 °C
    - 8 hours at 2–8 °C

    WARNING Protect the diluted bead suspension from light. Some of the dyes used to manufacture the beads are very light sensitive. Fluorescence levels can change if the beads are exposed to direct light for longer than 20 minutes.

4. PROCEDURE

Generate new Application Settings using BD OneFlow™ Setup Beads and LWB at the following times:

- Once a month to ensure consistent and accurate assay-specific PMTV setup
- Each time a new lot of BD OneFlow™ Setup Beads is used
- Each time a new lot of BD FACSDiva™ CS&T IVD Beads is used
- Whenever a new baseline is defined using BD FACSDiva™ CS&T IVD Beads
- After cytometer maintenance or service is performed

Reagents and Materials

Reagents and materials provided

- One vial of BD OneFlow™ Setup Beads, sufficient for 25 tests
- Monthly MFI target range card

    The monthly MFI target range card contains MFI ranges for all fluorescence detectors.
- Daily MFI target range card

    The daily MFI target range card contains MFI ranges for all fluorescence detectors that are optimized for optional daily monitoring. See the *Instrument Setup Guide for BD OneFlow™ Assays* for more information.

Reagents and materials required but not provided

- Installer with OneFlow™ Setup template (Catalog No. 659305)

    The template contains two global worksheets (*BD OneFlow™ TMFI Setup* and *BD OneFlow™ Scatter Setup*). Be sure to order this installer prior to using the BD OneFlow™ Setup Beads for the first time.
- Vortex mixer
- Pasteur pipets
- Micropipettor with tips
- 12 x 75-mm capped polystyrene tubes

- BD FACSFlow™ Sheath Fluid (Catalog No. 342003)
- BD FACSCanto™ II flow cytometer with a 4-2H-2V optical configuration

  See the cytometer user's guide for information.
- BD FACSDiva™ software v8.0.1 or later

  See the *BD FACSDiva™ Software Reference Manual*.
- BD FACSDiva™ CS&T IVD Beads (Catalog No. 656046 or 656047)

  See the BD FACSDiva™ CS&T IVD Beads IFU.
- Lysed washed blood (LWB) specimen from a normal donor

  Use the blood specimen within 24 hours of collection. See Lysing the blood specimen for instructions.
- BD FACS™ Lysing Solution(Catalog No. 349202)

  For dilution instructions and warnings, see the reagent IFU.
- Wash buffer (filtered PBS with 0.5% BSA and 0.09% sodium azide)

Installing the OneFlow™ Setup template

1. Insert the installer into the drive and click the installer icon.
2. Follow the prompts to install the template.

   The installer will copy and paste the template into the folder: D:\BDExport\Templates\Panel\BDPanels.

Lysing the blood specimen

You will use a LWB specimen to adjust FSC and SSC voltages.

WARNING All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection[1,2] and dispose of with proper precautions in accordance with federal, state, and local regulations. Never pipette by mouth. Wear suitable protective clothing, eyewear, and gloves.

1. Add 100 µL of whole blood from a normal donor to a tube labeled *LWB*.
2. Add 2 mL of 1X BD FACS™ Lysing Solution.
3. Vortex 3–5 seconds to mix well.
4. Incubate for 10 minutes at 18–25 °C.
5. Centrifuge at 540*g* for 5 minutes at 20–25 °C.
6. Remove the supernatant without disturbing the cell pellet and leave approximately 50 µL of residual liquid in the tube.
7. Vortex 3–5 seconds to resuspend the cell pellet.
8. Add 2 mL of wash buffer to the tube.
9. Vortex 3–5 seconds to mix well.
10. Centrifuge at 540*g* for 5 minutes at 20–25 °C.

11. Remove the supernatant without disturbing the cell pellet and leave approximately 50 µL of residual liquid in the tube.
12. Vortex 3–5 seconds to resuspend the cell pellet.
13. Add 250 µL of wash buffer to the tube.
14. Vortex 3–5 seconds to mix well.
15. Save the LWB sample to adjust FSC and SSC voltages. See Adjusting FSC and SSC on page 7.
16. Store at 2–25 °C until acquisition.

Preparing BD OneFlow™ Setup Beads

Before preparing BD OneFlow™ Setup Beads, verify that the daily performance check for the 4-2H-2V configuration was completed today and passed.

1. Label a 12 × 75-mm capped polystyrene tube *Setup beads*.
2. Thoroughly mix the BD OneFlow™ Setup Beads vial.
3. Prepare the diluted beads according to Table 1 and the task you are performing.

Table 1 BD OneFlow™ Setup Beads preparation

Task	BD FACSFlow™ Sheath Fluid (µL)	Beads (number of drops)
First time setup	700	2
Monthly setup	350	1

4. Return the BD OneFlow™ Setup Beads to 2–8 °C storage.
5. Vortex the tube gently before use.

   If not acquiring immediately, store the diluted beads, protected from light, for up to:

   - 1 hour at 18–25 °C
   - 8 hours at 2–8 °C

Setting up the software

1. In the BD FACSDiva™ workspace title bar, confirm that the 4-2H-2V optical configuration is selected.
2. From the menu bar, select **Experiment** > **New Experiment** > **Blank Experiment**, then click **OK**.
3. If prompted by the CST Mismatch dialog, select **Use CST Settings**.
4. Rename the experiment with the run date appended with OneFlow (for example, OneFlow Setup_today's date).
5. From the menu bar, select **Experiment** > **New Specimen**.

   The **Panel Template** window opens.
6. Click the **BD Panels** tab and select the **OneFlow™ Setup** template, then click OK.
7. Click **Cytometer Settings** in the Browser window.

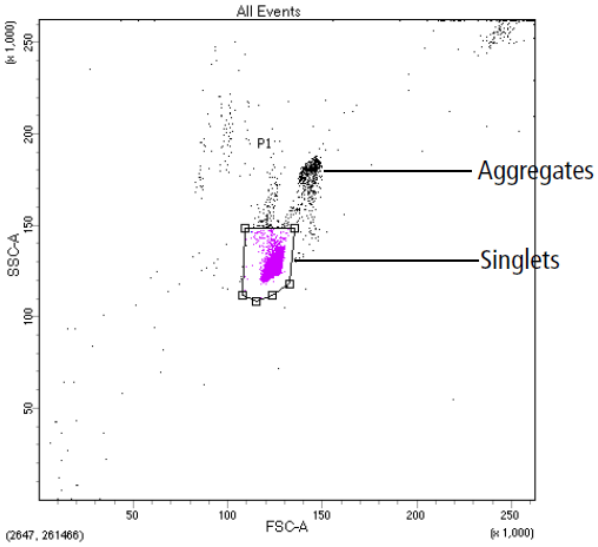
8. In the Inspector, select the **Parameters** tab and ensure that **FSC-A**, **FSC-H**, **SSC-A**, and **SSC-H** are all selected.
9. Navigate to the **Compensation** tab in the Inspector and deselect the **Enable Compensation** option.

Adjusting PMTVs

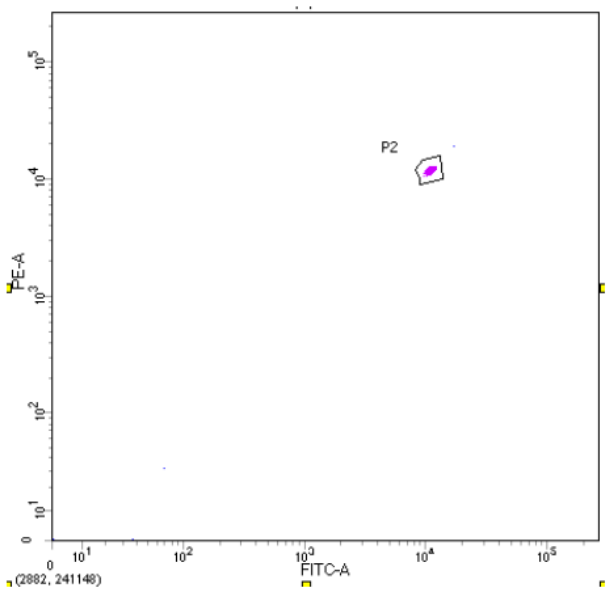
1. In the **Browser**, set the current tube pointer to the BD OneFlow™ Setup Beads tube.
2. In the Acquisition Dashboard, set **Events To Record** to 5,000.
3. Vortex the beads tube.
4. Install the tube on the cytometer.
5. Adjust the flow rate to **Low**, and click **Acquire Data**.

   NOTE It may take 10–15 seconds until events begin to appear.
6. In the FSC-A vs SSC-A dot plot, adjust the **P1** gate to include only the singlet bead population (no aggregates).

   NOTE Click the **Increase** button in the **Tools** menu of the global worksheet to see more detail in the FSC-A vs SSC-A dot plot.



7. In the FITC-A vs PE-A dot plot, adjust the **P2** gate to include only the singlet bead population.



8. In the Cytometer window, select the **Parameters** tab and adjust the voltages for FITC, PE, PerCP-Cy5.5, PE-Cy7, APC, APC-H7, V450, and V500 so that the MFI of the bead population in the P2 gate falls within the corresponding range on the monthly MFI target range card (Figure 1).

Figure 1 Example monthly MFI target range card

BD OneFlow™ Setup Beads (Monthly)

REF LOT

Fluorophore	Min (-2%)	TMFI	Max (+2%)
FITC	10397	10610	10822
PE	11896	12139	12382
PERCP-CY5.5	46584	47535	48486
PE-CY7	22194	22647	23100
APC	57164	58331	59497
APC-H7	129387	132028	134668
V450	9639	9835	10032
V500-C	24076	24568	25059

Monthly Target Ranges 23-16178-00

9. If needed, increase the size of the **P2** gate to ensure that the singlet bead population remains within the gate while adjusting the PMTVs.

Experiment Name:	OneFlow Setup_20140627	CYTOMETER CONFIG CRE...	2007-01-02T12:00:00-08:00
Specimen Name:	PMT Setup	CST PERFORMANCE EXPI...	2014-06-24T11:57:03-07:00
Tube Name:	OneFlow Setup Bead_001	CST REGULATORY STAT...	CE-IVD Performance Check
Record Date:	Jun 23, 2014 3:13:44 PM	CST BEADS EXPIRED:	False
CYTOMETER CONFIG NA...	3-laser, 8-color (4-2H-2V) (B...		

Population	FITC-A Median	PE-A Median	PerCP-Cy5-5-A Median	PE-Cy7-A Median
P2	10,654	12,196	47,223	22,513

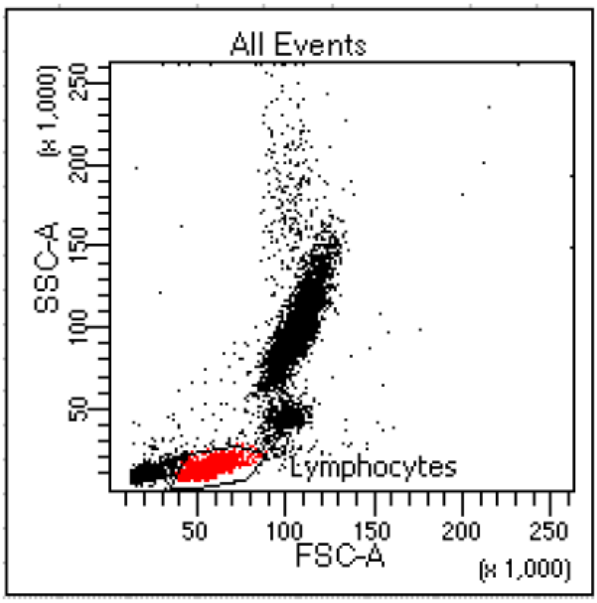
Population	APC-A Median	APC-H7-A Median	V450-A Median	V500-A Median
P2	58,579	132,245	9,751	24,481

10. Click **Record Data**.
11. Verify that the MFI values fall within range.

Adjusting FSC and SSC

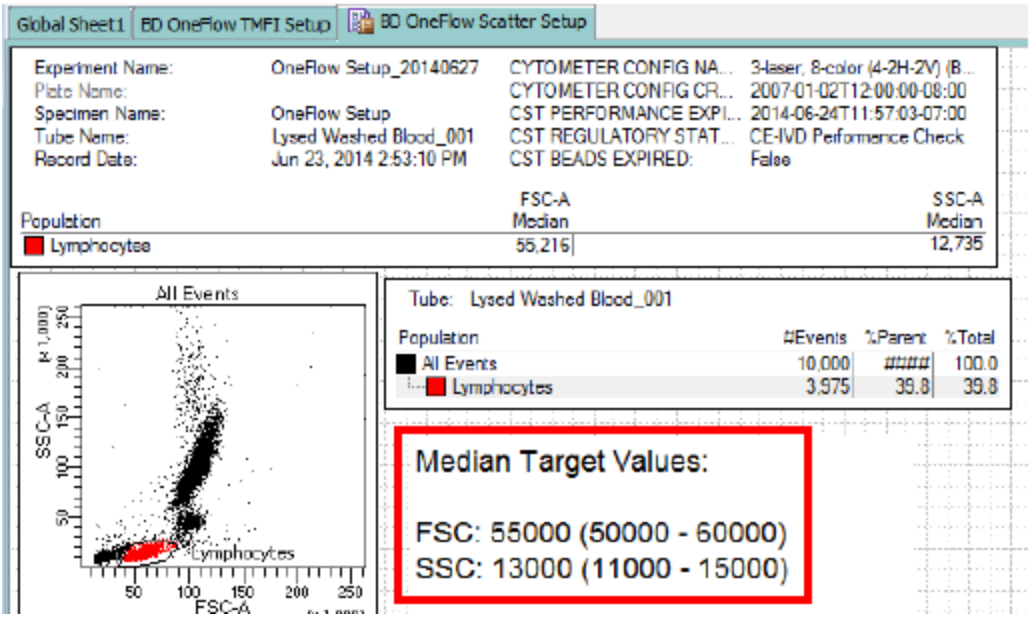
NOTE Use the normal LWB sample that you prepared for this procedure.

1. In the **Browser**, select the current tube pointer for the LWB tube.
2. In the Acquisition Dashboard, confirm that the **Events To Record** are set to 10,000 total events.
3. Vortex the LWB tube.
4. Install the LWB sample on the cytometer and confirm that the flow rate is set to **Low**.
5. Click **Acquire Data**.
6. In the Cytometer window, select the **Parameters** tab and lower the voltages for FSC and SSC so that the lymphocyte population is on scale.
7. In the Cytometer window, select the **Threshold** tab and set the FSC threshold to 10,000.



8. Adjust the Lymphocyte gate to encompass the entire lymphocyte population in the FSC vs SSC dot plot.
9. Adjust the FSC and SSC voltages to place the lymphocyte population within the FSC-A and SSC-A target value ranges given on the BD OneFlow™ Scatter Setup worksheet.

Figure 2 Statistics view on worksheet



10. If needed, re-adjust the lymphocyte gate.
11. Click **Record Data**.
12. Verify that the MFI values fall within range.
13. Right-click **Cytometer Settings** > **Application Settings** > **Save**, and click **OK**.

    CAUTION Use the default name for the Application Settings. Do not rename the Application Settings.

14. When prompted, click **Yes** to maintain the modified threshold values.

5. LIMITATIONS

- BD OneFlow™ Setup Beads are intended to set voltages appropriate for the BD multicolor tube assay when used with a BD FACSCanto™ II flow cytometer set with the 4-2H-2V optical configuration and BD FACSDiva™ software v8.0.1 or later.
- The PMT voltages and Application Settings generated using the BD OneFlow™ Setup Beads are intended to be used for the BD multicolor tube assay and should not be used for any other clinical reagents or assays.
- BD OneFlow™ Setup Beads do not perform as a fluorescence calibrator and should not be used for setting up a flow cytometer for quantitative fluorescence measurements.

6. PERFORMANCE CHARACTERISTICS

Performance of the BD OneFlow™ Setup Beads was established by testing at BD Biosciences laboratories in San Jose, California.

Accuracy

Accuracy testing was performed using BD FACSDiva™ software v8.0.1 or later on BD FACSCanto™ II flow cytometers using BD OneFlow™ Setup Beads (test method), Sphero™ Rainbow calibration particles (reference method), and BD® FC Beads (used as stable fluorescent particles). On each cytometer, detector gain settings were generated using BD OneFlow™ Setup Beads and Sphero Rainbow calibration particles by placing the beads within the bead lot-specific target MFI ranges specified for each detector. BD® FC Beads were acquired using each gain setup generated with the test and reference methods. Average MFI of the positive BD® FC Beads were compared between the test and reference methods. Data is shown in Table 2.

Table 2 Accuracy of MFI values between test and reference methods (relative mean bias)

Channel	% Relative bias	SD[a]
FITC	–0.30	1.01
PE	–0.31	1.40
PerCP-Cy5.5	0.42	0.70
PE-Cy7	1.69	1.03
APC	2.44	0.88
APC-H7	2.25	2.36
V450	–4.41	0.64
V500	0.27	0.39

a. SD= Standard deviation

Precision

Precision testing was performed using BD FACSDiva™ software v8.0.1 or later on multiple BD FACSCanto™ II flow cytometers using multiple lots of BD OneFlow™ Setup Beads over multiple days. BD® FC Beads were used as stable fluorescent particles. Detector gain settings were generated using BD OneFlow™ Setup Beads by placing the beads within the bead lot-specific target MFI ranges specified for each detector. Using the PMT gain settings generated for each setup, the eight single color BD® FC Beads were acquired. Percent CV of the MFI values of the positive BD® FC Beads were used to verify precision. Data is shown in Table 3.

Table 3 BD OneFlow™ Setup Beads precision (lot to lot and instrument to instrument)

Channel	%CV[a]	UCL[b]
FITC	8.6	10.2
PE	3.4	4.0
PerCP-Cy5.5	17.0	20.2

Channel	%CV[a]	UCL[b]
PE-Cy7	3.9	4.7
APC	1.3	1.5
APC-H7	2.8	3.3
V450	17.1	20.3
V500	4.8	5.7

a. CV = Coefficient of variation

b. UCL = Upper confidence limit of the 95% confidence interval

7. TROUBLESHOOTING

Problem	Possible Cause	Solution
No beads detected	Beads not mixed prior to diluting	Vortex the beads vial, prepare a fresh suspension of beads according to Table 1, and re-run the tube.
	Beads too dilute	
	Debris in the beads suspension	
	Incorrect beads used	
	Air bubbles in the flow cell or sheath filter	Check the fluidics for bubbles and debris. See the cytometer IFU for more information.
	Clogs within the sample tubes and lines	Check the fluidics for clogs and debris. See the cytometer IFU for more information.
	Back pressure in the waste lines	Check the waste tank vent for obstructions. See the cytometer IFU for more information.
	High scatter noise (FSC or SSC)	Perform monthly maintenance. See the cytometer IFU for more information. Call BD Biosciences.
	FSC threshold is set too high	Lower the FSC threshold.
	FSC and SSC PMTVs are not optimum	Optimize FSC and SSC PMTVs.

Problem	Possible Cause	Solution
No cells detected in lysed, washed blood sample	Air bubbles in the flow cell or sheath filter	Check the fluidics for bubbles and debris. See the cytometer IFU for more information.
	Clogs within the sample tubes and lines	Check the fluidics for clogs and debris. See the cytometer IFU for more information.
	Back pressure in the waste lines	Check the waste tank vent for obstructions. See the cytometer IFU for more information.
	Cell concentration in prepared samples is too low	Prepare a new sample.
	FSC and SSC PMTVs not optimum for cells	Optimize FSC and SSC PMTVs.

REFERENCES

1. *Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline—Fourth Edition*. Wayne, PA: Clinical and Laboratory Standards Institute; 2014. CLSI document M29-A4.
2. Centers for Disease Control and Prevention. 2007 Guideline for Isolation Precautions: Preventing Transmission of Infectious Agents in Healthcare Settings. https://www.cdc.gov/infectioncontrol/guidelines/isolation/index.html. Accessed March 12, 2019.

NOTICE

EU Only: Users shall report any serious incident related to the device to the Manufacturer and National Competent Authority.

Outside EU: Contact your local BD representative for any incident or inquiry related to this device.

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.

PATENTS AND TRADEMARKS

For US patents that may apply, see bd.com/patents.

HISTORY

Revision	Date	Changes made
23-15758(01)	2022-04	Updated to meet requirements for Regulation (EU) 2017/746.
23-15758(02)	2023-07	Updated legal manufacturer address. Added EU and Swiss importer addresses and importer symbol. Updated symbols glossary and Patents and Trademarks section.

Symbols Glossary

Please refer to product labeling for applicable symbols.

Symbol	Meaning
	Manufacturer
EC REP	Authorized representative in the European Community
CH REP	Authorised representative in Switzerland
	Date of manufacture
	Use-by date
LOT	Batch code
REF	Catalogue number
SN	Serial number
STERILE	Sterile
STERILE A	Sterilized using aseptic processing techniques
STERILE EO	Sterilized using ethylene oxide
STERILE R	Sterilized using irradiation
STERILE	Sterilized using steam or dry heat
	Do not resterilize
	Non-sterile
	Do not use if package is damaged and consult *instructions for use*
STERILE	Sterile fluid path
STERILE EO	Sterile fluid path (ethylene oxide)
STERILE R	Sterile fluid path (irradiation)
	Fragile, handle with care
	Keep away from sunlight
	Keep dry
	Lower limit of temperature
	Upper limit of temperature
	Temperature limit
	Humidity limitation
	Biological risks
	Do not re-use
	Consult *instructions for use* or consult electronic *instructions for use*
	Caution
LATEX	Contains or presence of natural rubber latex
IVD	In vitro diagnostic medical device
CONTROL -	Negative control
CONTROL +	Positive control
	Contains sufficient for <*n*> tests
	For IVD performance evaluation only
	Non-pyrogenic
#	Patient number
	This way up
	Do not stack

Symbol	Meaning
	Single sterile barrier system
PHT DEHP BBP	Contains or presence of phthalate: combination of bis(2-ethylhexyl) phthalate (DEHP) and benzyl butyl phthalate (BBP)
	Collect separately Indicates separate collection for waste of electrical and electronic equipment required.
CE	CE marking; Signifies European technical conformity
	Device for near-patient testing
	Device for self-testing
Rx Only	This only applies to US: "Caution: Federal Law restricts this device to sale by or on the order of a licensed practitioner."
CC	Country of manufacture "CC" shall be replaced by either the two letter or the three letter country code.
	Collection time
	Cut
	Peel here
12	Collection date
	Keep away from light
H_2	Hydrogen gas is generated
	Perforation
	Start panel sequence number
	End panel sequence number
	Internal sequence number
/	<Box #> / <Total Boxes>
MD	Medical device
	Contains hazardous substances
	Ukrainian conformity mark
FC	Meets FCC requirements per 21 CFR Part 15
c UL us	UL product certification for US and Canada
UDI	Unique device identifier
	Importer
	Place patient label in framed area only
MR	Magnetic resonance (MR) safe
MR	Magnetic resonance (MR) conditional
MR	Magnetic resonance (MR) unsafe
For use with	For use with
This Product Contains Dry Natural Rubber	This Product Contains Dry Natural Rubber
For Export Only	For Export Only
Instruments	Instruments

Note: Text layout in symbols is determined by label design.

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