ENGLISH

Stem-Comp™ Reagent

CE IVD REF IM3631

For In Vitro Diagnostic Use

50 tests – 20 µL / test – 1 mL

Stem-Comp Reagent: Reagent for adjusting color compensation settings on flow cytometers for CD34+ cell analysis

1. Intended Use

The Stem-Comp Reagent consists of a two color fluorescent reagent implementing two monoclonal antibodies. The antibodies are conjugated to fluorescein isothiocyanate (FITC) for the fluorescence 1 channel (FL1) of a flow cytometer and R Phycoerythrin (PE) for the fluorescence 2 channel (FL2). The Stem-Comp Reagent is used to adjust color compensations for automated or manual analysis with the Stem-Kit™ Reagents (Ref. IM3630).
Refer to the Stem-Kit Reagents package insert for complete instructions for use if performing analysis without stemONE™ System Software (Ref. 6915452).
Refer to the stemONE System Guide provided with the stemONE System Software for complete instructions for automated analysis.

2. Reagent Contents

The concentration of non-antibody reagents is 2 mg/mL bovine serum albumin (BSA), phosphate-buffered saline (PBS: 0.01 M sodium phosphate, 0.145 M sodium chloride, pH 7.2), and 0.1% sodium azide (NaN_3).

Fluorescence

- Fluorescein isothiocyanate (FITC) :
  - Excitation wavelength: 488 nm
  - Maximum emission wavelength: 525 nm
  - Main emission color: green
- R Phycoerythrin (PE):
  - Excitation wavelength: 488 nm
  - Maximum emission wavelength: 575 nm
  - Main emission color: orange-red

3. Statement of Warnings

1. This reagent contains 0.1% sodium azide. Sodium azide under acidic conditions yields hydrazoic acid, an extremely toxic compound. Azide compounds should be flushed with running water while being discarded. These precautions are recommended to avoid deposits in metal piping in which explosive conditions can develop. If skin or eye contact occurs, wash excessively with water.
2. Specimens, samples and all material coming in contact with them should be handled as if capable of transmitting infection and disposed of with proper precautions.
3. Never pipet by mouth and avoid contact of specimens, samples, or reagents with skin and mucous membranes.
4. Do not use reagent beyond the expiration date on the vial label.
5. Minimize exposure of reagent to light during storage or incubation.
6. Use Good Laboratory Practices (GLP) when handling this reagent.

4. Storage Conditions and Stability

- Store this reagent at 2 – 8°C in the dark.
- Opened vial must be capped tightly and stored at 2 – 8°C after use.
- Minimize exposure to light.
- Do not freeze.
- Reagent stability: this reagent is stable up to the expiration date printed on the vial label when stored unopened at 2 – 8°C. The open vial stability is 30 days.

4.1 Evidence of Deterioration

Any change in the physical appearance of the reagent (clear, slightly pink to reddish liquid) or any major variation in values obtained for control samples may indicate deterioration and the reagent should not be used.

4.2 Reagent Preparation

No preparation is necessary. Stem-Comp Reagent is used directly from the vial. Bring reagent to 18 – 25°C prior to use.

5. Materials Required but not Supplied

1. Deionized water.
2. Stem-Kit Reagents (Ref. IM3630) including 7-AAD Viability Dye.
3. Stem-Trol™ Control Cells (Ref. IM3632).
4. Plastic test tubes (12 x 75 mm).
5. Calibrated positive displacement pipette (20 µL, 100 µL) and tips or calibrated repeater pipette (20 µL, 100 µL, 2 mL) and tips.
6. Calibrated standard pipette (20 µL, 100 µL, 2 mL) and tips.
7. Vortex mixer.
8. Timer.
9. Flow cytometer.
10. StemONE Software (Ref. 6915452) ONLY for automated analysis of samples on COULTER® EPICS® XL™/XL-MCL™ flow cytometers equipped with System II™ Software (Version 3.0).

6. Procedure

Refer to the stemONE System Guide provided with the stemONE Software (Ref. 6915452) or refer to the Stem-Kit Reagents (Ref. IM3630) package insert for complete instructions for use.
Use the following procedure, when not using the stemONE System Software:

Sample Preparation for FL1 and FL2 compensation when 7-AAD Viability Dye is present:

1. Prepare the 7-AAD Compensation tube:
   a) Label one tube: 7-AAD COMP.
   b) Pipet 20 µL of Stem-Comp Reagent into the tube.
   c) Pipet 20 µL of 7-AAD Viability Dye into the tube.
   d) Using a positive displacement or repeater pipette, pipet 100 µL of well-mixed, fresh, normal whole blood into the tube.
   Important: there is a risk of incomplete lysis if blood specimen remains on the top or side of the test tube. Use care when pipetting to prevent the blood specimen from touching the top or side of the test tube. Clean the tube with a cotton swab, if necessary, to remove all traces of blood specimen from the top or side of the test tube.
   e) Vortex the tube for 5 seconds.
   f) Pipet 20 µL of vortexed Stem-Trol Control Cells into the tube.
   g) Immediately vortex the tube for 5 seconds.
2. Incubate the tube at room temperature (18 – 25°C) for 20 minutes, protected from light.
3. After incubation, add 2 mL of NH_4Cl Lysing Solution prepared at 1X to the tube. Refer to the stemONE System Guide (StemONE Software Ref. 6915452) or to the Stem-Kit Reagents (Ref. IM3630) package insert for the 1X NH_4Cl Lysing Solution preparation.
4. Vortex the tube for 5 seconds.
5. Incubate the tube at room temperature (18 – 25°C) for 10 minutes, protected from light.

Prepared samples can be stored at room temperature protected from light if flow cytometry analysis occurs within 2 hours of preparation. Otherwise, cover the sample and store at 2 – 8°C protected from light.

Setting Color Compensation:

1. Ensure the flow cytometer has been properly aligned and standardized.
2. Analyze samples with standardized high voltage and gain settings used for your multicolor application.
3. Create a two-parameter Forward Scatter *vs.* Side Scatter histogram with a region (A) set around the lymphocyte population, and a second, larger region (B) set around the Stem-Trol Control Cells population (see Figure 1).
4. Create 3 two-parameter log fluorescence histograms:
   FL2 *vs.* FL1
   FL2 *vs.* FL4
   FL1 *vs.* FL4
   - The FL2 *vs.* FL1 histogram displays events from the lymphocyte region.
   - The FL2 *vs.* FL4 and FL1 *vs.* FL4 histograms display events from the Stem-

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Trol Control Cell region. Ensure that the two-parameter log fluorescence histograms represent all the fluorochrome combinations used in your multicolor analysis (See Figure 2).

5. Analyze the sample and adjust the appropriate color compensation settings until the "X" mean intensities of the population in quadrants 1 and 3 are equal (±0.1) and until the "Y" mean intensities in quadrants 3 and 4 are equal (±0.1).
6. Transfer the established color compensation settings to the protocol used in your multicolor analysis.

EXAMPLE DATA

Figure 1 is a two-parameter histogram of Forward Scatter *vs.* Side Scatter representing lysed, normal, fresh whole blood premixed with Stem-Trol Control Cells (Ref. IM3632), then stained with Stem-Comp Reagent (Ref. IM3631). Acquisition is performed on a COULTER EPICS XL/XL-MCL flow cytometer. Analysis is performed with System II Software (Version 3.0).

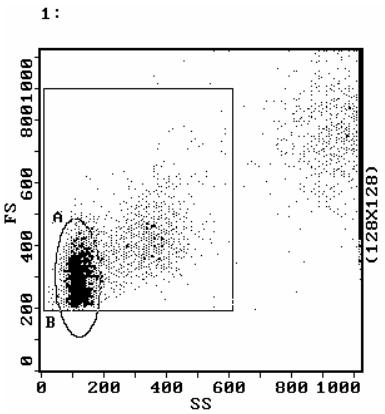

Figure 1: Forward Scatter *vs*. Side Scatter Histogram

Figure 2 includes two parameter log fluorescence histograms (Fluorescence Intensity *vs.* Fluorescence Intensity). The histograms (i.e. Histogram 2 for FL2 *vs.* FL1, Histogram 3 for FL2 *vs.* FL4, and Histogram 4 for FL1 *vs.* FL4) are representations of lysed, normal, fresh whole blood premixed with Stem-Trol Control Cells (Ref. IM3632), then stained with Stem-Comp Reagents (Ref. IM3631) and 7-AAD. Acquisition is performed on a COULTER EPICS XL/XL-MCL flow cytometer. Analysis is performed with System II Software (Version 3.0).

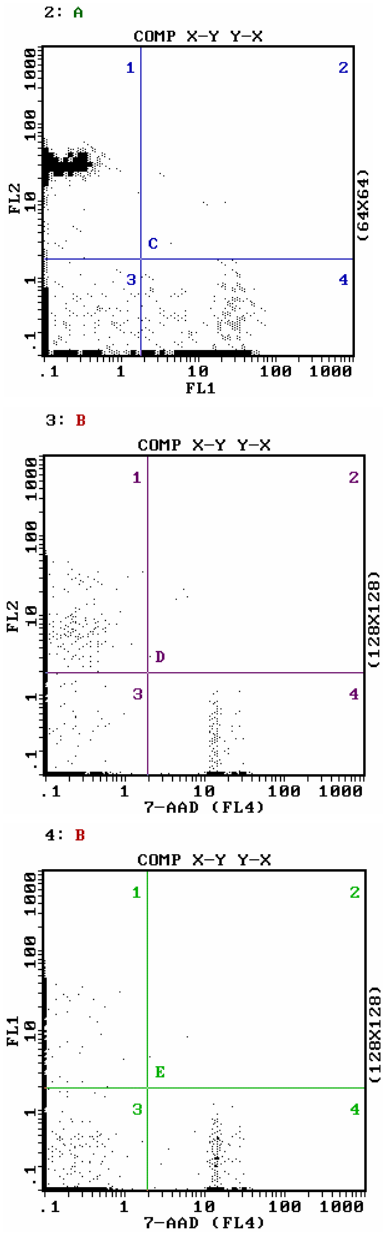

Figure 2: Two Parameter Log Fluorescence Histograms

TRADEMARKS

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