STEM CELL
LABORATORY (STCL)

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DOCUMENT TITLE:

Color Compensation Settings for BD FACSCanto II

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Control Information

Author: WATER002	**Owner:** WATER002
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Follow this procedure to reset compensation settings after service/maintenance, if compensation values are out of range, or when checking daily QC if populations deviate from original gates.

NOTE: Will need 12 x 75 mm plain tubes (***NOT*** *TruCount tubes*)
- **Tube C-1:** Unstained
- **Tube C-2**: Add 10uL CD3 FITC (BD Cat. No. 349201)
- **Tube C-3**: Add 2.5uL CD3 PE (BD Cat. No. 340662)
- **Tube C-4**: Add 7uL CD3 PerCP (BD Cat. No. 340663)
- **Tube C-5**: Add 2.5uL CD8 APC (BD Cat. No. 340659)
- **Tube C-6**: Add 15uL 7AAD only (BD Cat. No. 559925 or 51-68981E)

Staining Protocol:
- Add appropriate amounts of antibody to each respective tube (*amounts included above*)
- From Status Flow Cells, Lymphs
- Add 50uL to each tube
- Vortex samples
- Incubate at room temperature (*in the dark*) for 15 minutes
- Lyse, using 450uL/tube, with PharmLyse (BD Cat. No. 555899; using 10% stock solution
  - To prepare the 10% stock solution, add 1-part PharmLyse to 9-parts of sterile, distilled, filtered water from the deionized water system labeled "**spigot # 1**".
- Incubate at room temperature (*in the dark*) for 15 minutes

Acquisition Protocol:
Diva software:
- Acquire in Comp 4-Color Template for 7AAD or PerCP (*these fluorochromes are in same channel*)
- Apply application settings before running comps
- Link to previous comp experiment
- Must acquire using Global Worksheet (WS)
- Normal WS will automatically be created when "create compensation controls"
- When start new CST bead lot, we must update application settings, which could affect comps
- Check the cytometer settings by adding an extra specimen tube (not part of comp controls) and don't record, just acquire a few events for each parameter to see if population looks good and is on scale
- Check FSC/SSC settings at beginning of run before setting up carousel; otherwise, will run whole carousel at settings for first tube before they're adjusted
- Save new comp settings and overwrite the old 4-color comp template with new ones (<u>NOTE</u>: It is not recommended by BD to try and save the old settings as this could cause confusion in choosing the incorrect settings)
- Check settings daily when acquiring Status Flow Controls.

Signature Manifest

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