INSTRUCTIONS

Cellomics® Whole Cell Stains Blue, Green, Orange and Red

For High Content Screening and Fluorescence Microscopy

1998.1

Number	Description	Storage	Ex/Em
8403501 **8403502**	**Whole Cell Stain Blue,** 1 × 96 **Whole Cell Stain Blue,** 5 × 96	4°C	350/450 nm
8403201 **8403202**	**Whole Cell Stain Green,** 1 × 96 **Whole Cell Stain Green,** 5 × 96	-20°C	493/518 nm
8403301 **8403302**	**Whole Cell Stain Orange**,1 × 96 **Whole Cell Stain Orange,** 5 × 96	4°C	550/568 nm
8403401 **8403402**	**Whole Cell Stain Red,** 1 × 96 **Whole Cell Stain Red,** 5 × 96	4°C	654/673 nm

Storage: Store the product as indicated above. Keep stains away from light and moisture. See the **Solution Preparation** section for storage and stability of prepared solutions.

Warning: Please completely read these instructions and the accompanying material safety data sheets before using this product. Cellomics Reagents are not for diagnostic use in humans or animals.

Introduction

The Cellomics Whole Cell Stains provide excellent staining for HCS assays and fluorescence microscopy. These stains are intense, highly photostable and match the output wavelengths of common fluorescence instrumentation (Figure 1). Effective image analysis in HCS cell-based assays and fluorescence microscopy requires fluorescent labeling of the entire cell. In these assays, the cellular primary object is used to identify and count individual cells and define the cell region in which the image analysis is applied. The primary object might be a major cellular component, such as the nucleus, a large organelle or the whole cell. When the whole cell is the primary object, high-quality Cellomics Whole Cell Stains effectively distinguish intact cells from bordering cells (Figure 2).

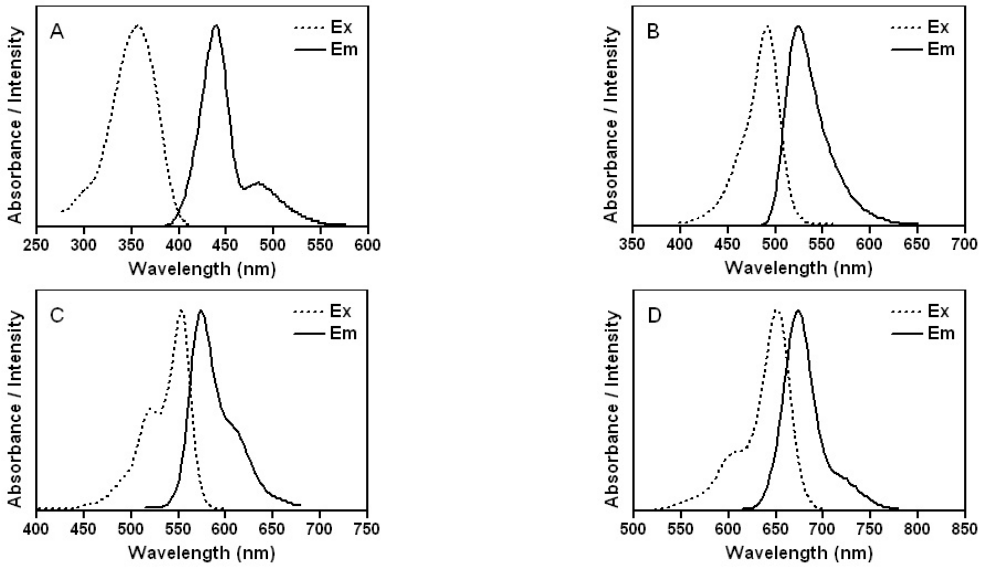

Figure 1. Excitation and emission spectra for the Cellomics Whole Cell Stains: Panel A blue stain in methanol, Panel B green stain in PBS, Panel C orange stain in PBS and Panel D red stain in PBS.

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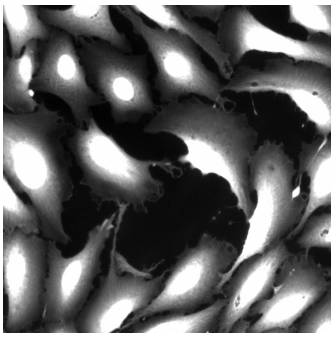

Figure 2. Image of human U2OS cells stained with Whole Cell Stain Green (with 20X objective). U2OS cells were stained for 30 minutes as described in the protocol. Whole Cell Stain Blue, Orange and Red stain the cell correspondingly.

Additional Materials Required

- Phosphate-buffered saline (PBS) (Thermo Fisher Scientific, Pierce Product No. 28372)
- Dimethylsulfoxide (DMSO)

Solution Preparation

Whole Cell Stain Stock Solution	For Whole Cell Stain Green, Orange and Red, add 50 µl of DMSO to the 1 × 96 vial or 250 µl DMSO to the 5 × 96 vial and mix. If there is any stain lodged under the cap, reconstitute it carefully using a pipette. For Whole Cell Stain Blue, add 100 µl of DMSO to the 1 × 96 vial or 500 µl DMSO for the 5 × 96 vial and mix. Discard unused Whole Cell Stain Stock Solution or for short-term storage (< 1 week), store at -20°C.
Whole Cell Stain Working Solution (per 96-well plate)	For Whole Cell Stain Green, Orange and Red, dilute 50 µl of the Whole Cell Stain Stock Solution to 5 ml with PBS and mix well. For Whole Cell Stain Blue, dilute 100 µl of the stain stock solution to 5 ml with PBS and mix well. **Note**: For best results, use the whole cell stain working solution immediately; however, it remains effective for at least 3 hours at room temperature. After staining, discard any unused stain working solution.

Whole Cell Staining Procedure

- Do not allow the wells to dry at any time during the protocol.
- Allow all vials to warm to room temperature before opening.
- Perform all steps at room temperature unless indicated otherwise.
- Using conditions other than those specified in these instructions may require protocol optimization.

1. Fix cells with formaldehyde at room temperature. If desired, probe with specific antibodies or dyes before using the Whole Cell Stain.

   Note: Cell permeabilization (e.g., 0.1% Triton X-100 in PBS for 15 minutes) may enhance staining intensity.

2. Rinse cells 2-3 times with PBS.
3. Add 50 µl of the Whole Cell Stain Working Solution to each well.
4. Incubate cells in the dark for 30 minutes at room temperature. Optimal staining time varies from 10 minutes to 3 hours depending on cell type.
5. Aspirate the Whole Cell Stain Working Solution and wash three times with PBS.
6. Seal plate and evaluate on a HCS reader. For fluorescence microscopy, observe the stained cells using the proper excitation and emission filters.
7. Store sealed plates in the dark at 4°C.

Recommendations for Automation

- Plating Cells: To improve the uniformity and throughput of plating cells, use a liquid-handling system such as a Titertek® Multidrop Dispenser.
- Dead Volumes: Every piece of automation instrumentation has a non-recoverable dead volume associated with it. Be aware of these dead volumes, priming volumes and rinsing volumes when calculating your reagent requirements.
- Nonspecific Binding: Because of the potential of reagent interaction with large surface areas inherent to tubing, syringes and peristaltic pumps, pre-priming with reagents or pre-coating with protein blockers may be warranted.
- Mixing: Gentle mixing may be required when adding a DMSO-based solution to keep overly concentrated solutions from lying on top of the cell layer. Be careful not to dislodge cells or beads during mixing procedures.
- Cell Washing: Use an automated plate washer designed to gently wash attached cells. Be careful not to dislodge cells or beads during cell washing.
- Incubation: Minimize the time when plates with live cells are out of a controlled CO_2 environment. For best results, use an automated incubator to deliver plates to a pipetting deck.
- Exposure: Minimize operator exposure to fixative by some form of containment. Some reagents and compounds are light-sensitive; be aware of these constraints when scaling up for an automated run.