



# CytoWatch™ Wash-free fluorescence cell imaging buffer \*10X\*

Catalog number: 20045, 20046 Unit size: 10 mL, 100 mL

Component	Storage		Amount (Cat No. 20046)
1.2.2.1	Freeze (< -15 °C), Minimize light exposure	10 mL	100 mL

## **OVERVIEW**

CytoWatch™ Wash-free fluorescence cell imaging buffer \*10X\* is a ready-to-use buffer optimized for fluorescence cell imaging. In some cases, this buffer significantly enhances the imaging signal. It is used in wash steps when performing immunohistochemistry (IHC) or immuno-labeling with tissue or 3D cell culture. CytoWatch™ Wash-free fluorescence cell imaging buffer is 10X concentrated and should be diluted to 1X with PBS before use.

#### SAMPLE EXPERIMENTAL PROTOCOL

## Typical Assay Protocol (for one 96-well plate

 Thaw 10X CytoWatch™ Wash-free fluorescence cell imaging buffer at room temperature before use.

**Note:** It is OK to use if the buffer has precipitates.

 Prepare a 1X CytoWatch™ Wash-free fluorescence cell imaging buffer by adding 1 mL of 10X CytoWatch™ Wash-free fluorescence cell imaging buffer to 9 mL of HHBS (1X Hank's with 20 mM Hepes buffer, pH 7.0, cat#20001) or a buffer of your choice, and mix well.

**Note:** 10 mL of 1X staining buffer is enough for one plate. The buffer is stable at room temperature. It is recommended to aliquot and store any unused 10X assay buffer at < -20 °C. Protect from light. Avoid repeated freeze-thaw cycles.

- 3. Add the cell staining dye stock solution (generally, a concentrated DMSO solution) into 1X CytoWatch™ Wash-free fluorescence cell imaging buffer (from Step 2) to make the final well concentration 2X of the desired concentration.
- 4. Add the 2X Assay Solution (from Step 3) to the microplate well, making sure it's the same volume as the cell culture medium (e.g.,  $100 \mu L/well/96$ -well or  $25 \mu L/well/384$ -well).
- 5. Incubate the cells in a 37 °C, 5% CO2 incubator, or as desired.

Note: The staining dye has the potential to disrupt the effectiveness of the 1X CytoWatch™ Wash-free fluorescence cell imaging buffer. If this occurs, it is advisable to utilize a preferred cell staining method and swap out the cell staining solution with either the cell growth medium or HHBS. Following this, add 100 µL/well/96-well (25 µL/well/ 384-well) of 1X CytoWatch™ Wash-free fluorescence cell imaging buffer into each respective well.

6. Observe the cells with a fluorescence microscope or a plate reader as required.

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