Rapid Labeling Protocol for 1nmol Janelia Fluor® and Janelia Fluor® JFX HaloTag® Ligands

Instructions for Use of Products HT1010, HT1020, HT1030, HT1040, HT1050, HT1060, HT1070, HT1100 and HT1110.

Promega

Quick Protocol

This protocol is intended for reconstituting aqueous-buffer-soluble 1nmol Janelia Fluor® and Janelia Fluor® JFX HaloTag® Ligands and labeling live cells.

Materials to Be Supplied by the User

- optical bottom chamber with cells expressing HaloTag® fusion protein
- complete culture medium appropriate for your cells, at 37°C
- confocal microscope or wide-field fluorescent microscope equipped with appropriate filter sets
- 37°C + CO_2 cell culture incubator
- **optional:** culture medium, lacking phenol red at 37°C

Protocol

1. Equilibrate a vial of 1nmol Janelia Fluor® or Janelia Fluor® JFX HaloTag® Ligand to room temperature.
2. Add 1ml of cell medium or chosen aqueous buffer.
3. Incubate HaloTag® Ligand in medium for 2–3 minutes with intermittent agitation to dissolve the ligand. Do **not** pipet or vortex to resuspend the ligand. This yields a 5X (1µM) working stock solution.
4. Add the 5X working stock solution to cells at a 1X final concentration of 200nM as a recommended starting point. Further optimizing of ligand concentration may be necessary (1).
5. Incubate the cells with the Janelia Fluor® or Janelia Fluor® JFX HaloTag® Ligand for 30 minutes at 37°C + CO_2 in a cell culture incubator (2,3).

   If using the 1nmol Janelia Fluor® 503 HaloTag® Ligand, incubate with cells for 1 hour at 37°C + CO_2 in a cell culture incubator (2).
6. Aspirate medium and replace with fresh cell medium. Alternatively, replace with phenol red-free medium to minimize background signal.
7. Transfer to a microscope and capture images.

Table 1. Excitation and Emission Maxima for Janelia Fluor® and Janelia Fluor® JFX HaloTag® Ligands.

Ligand	Excitation Maximum	Emission Maximum
Janelia Fluor® 503 HaloTag® Ligand	503	529
Janelia Fluor® 549 HaloTag® Ligand	549	571
Janelia Fluor® JFX554 HaloTag® Ligand	554	576
Janelia Fluor® 585 HaloTag® Ligand	585	609
Janelia Fluor® 635 HaloTag® Ligand	635	652
Janelia Fluor® 646 HaloTag® Ligand	646	664
Janelia Fluor® JFX650 HaloTag® Ligand	650	667

Notes:

a. Lower-expressing cells may require lower ligand concentrations that can enhance the signal-to-background ratio.

b. If using lower ligand concentrations, longer incubation times may be required to reach maximum intensity.

Additional protocol information is available in Technical Manual #TM260, online at: **www.promega.com**

References

1. Grimm, J.B., *et al*. (2015) A general method to improve fluorophores for live-cell and single-molecule microscopy. *Nat. Methods* **12**, 244–50.
2. Grimm, J.B., *et al*. (2017) A general method to fine-tune fluorophores for live-cell and in vivo imaging. *Nat Methods* **14**, 987–94.
3. Grimm J.B., *et al*. (2021) A general method to improve fluorophores using deuterated auxochromes. *JACS Au*. **1**, 690–6.