The Jackson Laboratory

HALOTAG® STAINING PROTOCOL

Janelia Fluor® 646 and Hoechst® 33342 staining for HaloTag® iPSC lines (96 well format)

REAGENT LIST

Promega #GA1120 — Janelia Fluor® 646 HaloTag® Ligand, 5 µg (solid)

LIGAND PREPARATION

1. Add **35.5 µl DMSO** to **5 µg Janelia Fluor® 646 HaloTag® Ligand (#GA1120)** to generate a **200 µM stock solution**.
2. **Do not freeze/thaw after resuspension**; prepare single use aliquots.
3. The Promega manual recommends **single use only**.
4. Prolonged storage in DMSO is not recommended, as it results in reduced ligand stability.

CELL STAINING FOR IMAGING (ADHERENT CELLS)

(60 wells of a 96 well plate)

MEDIA PREPARATION

Bring **6 ml StemFlex™** medium to room temperature.

WORKING STOCK PREPARATION

Prepare **1 µM HaloTag® Ligand working stock** by mixing:

- **1.5 µl** of 200 µM Janelia Fluor® 646 HaloTag® Ligand
- **300 µl** StemFlex™ medium

  (1:200 dilution; final concentration 1 µM)

STAINING MIX PREPARATION

Combine:

- **5.7 ml** StemFlex™ medium
- **300 µl** of 1 µM Janelia Fluor® 646 HaloTag® Ligand
- **12 µl** of 1,000 µg/ml Hoechst® 33342

Total volume: 6 ml

STAINING PROCEDURE

1. Remove culture medium.
2. Add **80–100 µl per well** of HaloTag® Ligand + Hoechst® staining mix.
3. Incubate **20 min at 37 °C**.
4. Aspirate and rinse once with **200 µl DPBS**.
5. Add **200 µl StemFlex™ medium** per well.
6. Image on **Opera Phenix, 60x, 0.5 µm z stack**.

CELL STAINING FOR FLOW CYTOMETRY (SUSPENDED CELLS)

1. Pellet **200,000 cells** in a 96 well V bottom plate.
2. Resuspend cells in **200 µl staining mix**:
   - 1.5 µl 200 µM Janelia Fluor® 646 HaloTag® Ligand
   - 300 µl StemFlex™ medium

   (Final concentration: 1 µM HaloTag® Ligand)
3. Incubate **30 min at 37 °C**.
4. Spin and wash **twice with PBS**.
5. Resuspend in **300 µl PBS/FBS**.
6. Acquire on flow cytometer using **APC Cy7 A channel**.

