

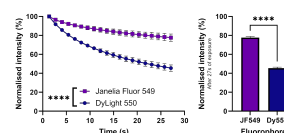
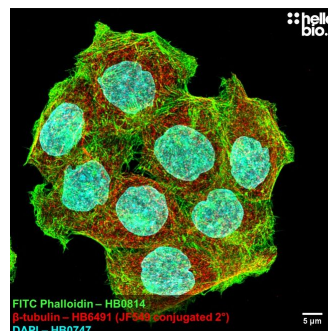
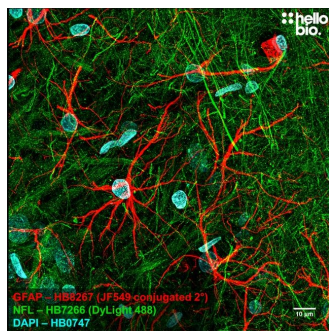
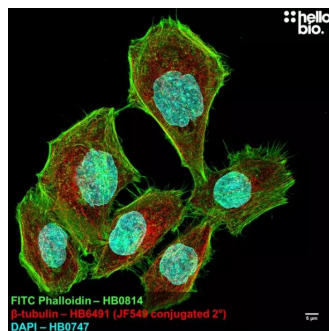
## DATASHEET

### Janelia Fluor® 549 NHS Ester (Succinimidyl Ester)

#### Product overview

<b>Name</b>	Janelia Fluor® 549 NHS Ester (Succinimidyl Ester)
<b>Cat No</b>	HB7336
<b>Biological description</b>	Cell-permeable, yellow fluorescent dye with an NHS ester (succinimidyl ester (SE)) reactive group. NHS esters react with primary amines on proteins and are commonly used for conjugating dyes to proteins, antibodies amine-modified oligonucleotides etc.
<b>Alternative names</b>	<b>Spectrally similar dyes:</b> Alexa Fluor® 546, Alexa Fluor® 555, BDY TMR-X, Atto 550, CF 555, TAMRA, Cyanine 3
<b>Biological action</b>	JF549, JF549-SE
<b>Description</b>	Dyes & stains Yellow dye for coupling to primary amine groups. Suitable for super resolution microscopy (e.g. dSTORM, STED), confocal microscopy, live cell imaging and flow cytometry.

#### Images



#### Biological Data

##### Application notes

##### #Protocol 1: Conjugation of Janelia® Fluor 549 SE to antibodies

- Using a desalting column, perform buffer exchange (following manufacturer instructions) of antibody into a carbonate buffer (100mM, pH 8-8.25)
- Mix together the antibody and Janelia® Fluor 549 SE (prepared at 10mM in anhydrous DMSO or DMF) in a 15:1 molar ratio. Incubate in the dark for 60 minutes at room temperature with gentle mixing.
- Add 10% by volume of 0.75M Tris-HCl pH7.4 (to a final concentration of 75mM) to stop the conjugation reaction. Incubate for 10-15 minutes at room temperature in the dark with gentle mixing.
- Use a desalting column, perform buffer exchange (following manufacture instructions) of antibody into PBS 0.05% sodium azide. This step also removes any unbound dye

## #Protocol 2: Immunocytochemistry

- ICC was performed upon 4% PFA fixed HeLa cells using **FITC Phalloidin** (1:500) and a anti- $\beta$  tubulin monoclonal antibody (HB6491, 1:2,000 / 0.5 $\mu$ g/ml). A polyclonal goat anti-mouse Janelia<sup>®</sup> Fluor 549 conjugated antibody was used at a dilution of 1:300 as a secondary antibody.
- Please see our detailed [immunocytochemistry protocol](#) for details of the full protocol.

## #Protocol 3: Immunohistochemistry

- 40 $\mu$ m horizontal sections were cut from a 4% PFA fixed rat brain.
- IHC(IF) was performed using mouse monoclonal anti-GFAP (**HB8267**, 1:1000 dilution / 1 $\mu$ g/ml) and rabbit monoclonal anti-NFL (**HB7266**, 1:2000 / 0.5 $\mu$ g/ml) antibodies. A polyclonal goat anti-mouse Janelia<sup>®</sup> Fluor 549 conjugated antibody was used at a dilution of 1:300 as a secondary antibody.
- Please see our detailed [immunohistochemistry protocol](#) for details of the full protocol

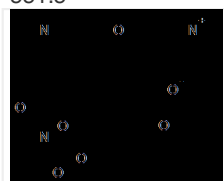
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## Solubility & Handling

<b>Storage instructions</b>	-20 °C
<b>Solubility overview</b>	Soluble in DMSO
<b>Handling</b>	This compound is light and temperature sensitive; exposure to light may affect compound performance. We therefore recommend storing the material in a freezer and protecting from light.
<b>Shipping conditions</b>	Ship on ice
<b>Important</b>	This product is for RESEARCH USE ONLY and is not intended for therapeutic or diagnostic use. Not for human or veterinary use

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## Chemical Data

<b>Chemical name</b>	3,6-Di-1-azetidiny-9-[2-carboxy-5-[(2,5-dioxo-1-pyrrolidinyloxy)carbonyl]phenyl]xanthylium, inner salt
<b>Molecular Weight</b>	551.5
<b>Chemical structure</b>	
<b>Molecular Formula</b>	C <sub>31</sub> H <sub>25</sub> N <sub>3</sub> O <sub>7</sub>
<b>CAS Number</b>	1811539-32-8
<b>PubChem identifier</b>	124201856
<b>SMILES</b>	<chem>C1CN(C1)C2=CC3=C(C=C2)C(=C4C=CC(=[N+]5CCCC5)C=C4O3)C6=C(C=CC(=C6)C(=O)ON7C(=O)CCC7=O)C(=O)[O-]</chem>
<b>Source</b>	Synthetic
<b>InChiKey</b>	DWUIXAGWHCMXHN-UHFFFAOYSA-N
<b>Licensing details</b>	Sold under license from the Howard Hughes Medical Institute, Janelia Research Campus

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## References

### A general method to improve fluorophores for live-cell and single-molecule microscopy.

Grimm JB et al (2015) Nature methods 12

**PubMedID** [25599551](#)

### A general method to fine-tune fluorophores for live-cell and in vivo imaging.

Grimm JB et al (2017) Nature methods 14

**PubMedID**

[28869757](#)

**Synthesis of Janelia Fluor HaloTag and SNAP-Tag Ligands and Their Use in Cellular Imaging Experiments.**

Grimm JB et al (2017) Methods in molecular biology (Clifton, N.J.) 1663

**PubMedID**

[28924668](#)

**Rational Design of Bioavailable Photosensitizers for Manipulation and Imaging of Biological Systems.**

Binns TC et al (2020) Cell chemical biology 27

**PubMedID**

[32698018](#)

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