Hello Bio, Inc.
304 Wall St., Princeton, NJ 08540 USA

T. 609-683-7500
F. 609-228-4994

customercare-usa@hellobio.com

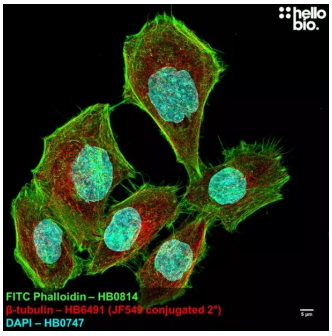
DATASHEET

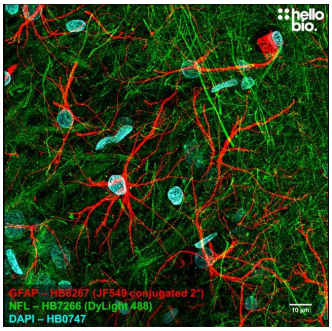
Janelia Fluor® 549 NHS Ester (Succinimidyl Ester)

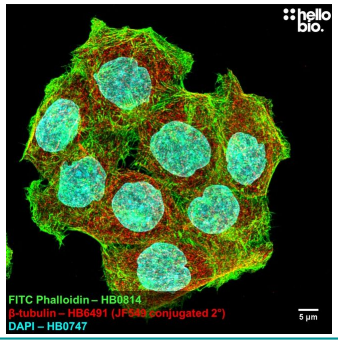
Product overview

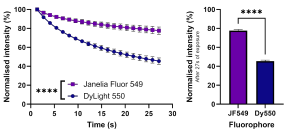
Name	Janelia Fluor® 549 NHS Ester (Succinimidyl Ester)
Cat No	HB7336
Biological description	Cell-permeable, yellow fluorescent dye with an NHS ester (succinimidyl ester (SE)) reactive group. NHS esters react with primary amines on proteins and are commonly used for conjugating dyes to proteins, antibodies amine-modified oligonucleotides etc. Janelia Fluor® 549 is 2 x brighter than TMR and Cy3 *in vitro* and live-cell experiments. Suitable for super resolution microscopy (SRM) including techniques such as STED and dSTORM (both live and fixed cells). May also be used for cellular imaging when combined with the HaloTag or SNAP-tag self labelling systems and confocal microscopy. **Spectrally similar dyes:** Alexa Fluor® 546, Alexa Fluor® 555, BDY TMR-X, Atto 550, CF 555, TAMRA, Cyanine 3
Alternative names	JF549, JF549-SE
Biological action	Dyes & stains
Description	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-β tubulin monoclonal antibody (HB6491, 1:2,000 / 0.5µg/ml). A polyclonal goat anti-mouse Janelia© 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.

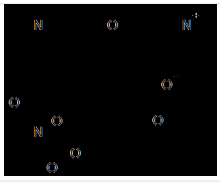
#Protocol 3: Immunohistochemistry

- 40µm horizontal sections were cut from a 4% PFA fixed rat brain.
- IHC(IF) was performed using mouse monoclonal anti-GFAP (HB8267, 1:1000 dilution / 1µg/ml) and rabbit monoclonal anti-NFL (HB7266, 1:2000 / 0.5µg/ml) antibodies. A polyclonal goat anti-mouse Janelia© 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

Solubility & Handling

Storage instructions	-20°C
Solubility overview	Soluble in DMSO
Handling	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.
Shipping conditions	Ship on ice
Important	This product is for RESEARCH USE ONLY and is not intended for therapeutic or diagnostic use. Not for human or veterinary use

Chemical Data

Chemical name	3,6-Di-1-azetidinyl-9-[2-carboxy-5-[[(2,5-dioxo-1-pyrrolidinyl)oxy]carbonyl]phenyl]xanthylium, inner salt
Molecular Weight	551.5
Chemical structure	
Molecular Formula	$C_{31}H_{25}N_3O_7$
CAS Number	1811539-32-8
PubChem identifier	124201856
SMILES	C1CN(C1)C2=CC3=C(C=C2)C(=C4C=CC(=[N+]5CCC5)C=C4O3)C6=C(C=CC(=C6)C(=O)ON7C(=O)CCC7=O)C(=O)[O-]
Source	Synthetic
InChiKey	DWUIXAGWHCMXHN-UHFFFAOYSA-N
Licensing details	Sold under license from the Howard Hughes Medical Institute, Janelia Research Campus

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