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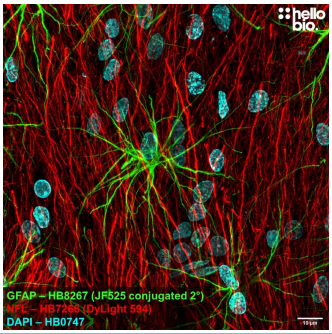
DATASHEET

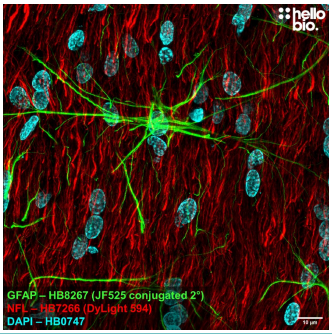
Janelia Fluor® 525 NHS Ester (Succinimidyl Ester)

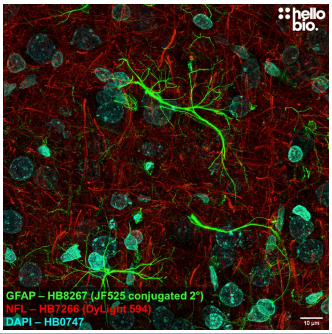
Product overview

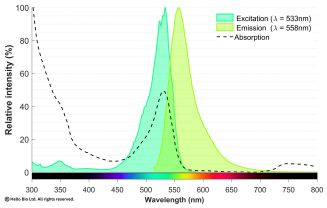
Name	Janelia Fluor® 525 NHS Ester (Succinimidyl Ester)
Cat No	HB8455
Biological description	Cell-permeable, yellow fluorescent dye with an NHS ester (succinimidyl ester (SE)) reactive group. NHS esters react with primary amines on proteins and are commonly used for conjugating dyes to proteins, antibodies amine-modified oligonucleotides etc. Suitable for super resolution microscopy (SRM) including techniques such as dSTORM (both live and fixed cells), cellular imaging when combined with the HaloTag or SNAP-tag self labelling systems and confocal microscopy. Can also be multiplexed with Janelia Fluor ® 635 SE for two color imaging. **Spectrally similar dyes:** Alexa Fluor® 532, Alexa Fluor® 514, Atto 532, CF514, CF532
Alternative names	JF525,SE
Biological action	Dyes & stains
Description	Yellow dye for coupling to primary amine groups. Suitable for super resolution microscopy (e.g. dSTORM), confocal microscopy and live cell imaging.

Images









Biological Data

Application notes

#Protocol 1: Conjugation of Janelia© Fluor 525 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 525 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: 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 525 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

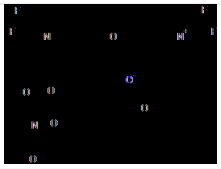
#Protocol 3: Measurement of excitation and emission spectra of Janelia Fluor ® 525, SE

- Janelia Fluor ® 525, SE was prepared at 1µm in PBS.
- Spectra were generated on a Tecan Infinite M200 PRO using the following parameters:
  - Excitation: Recording at 618nm while exciting between 280nm and 590nm
  - Emission: Exciting at 484nm while recording between 510nm and 800nm
  - Absorbance: Measured between 300 and 800nm

Solubility & Handling

Storage instructions	-20°
Solubility overview	Soluble in DMSO
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-(3,3-difluoroazetidinyl)-9-[2-carboxy-5-[[(2,5-dioxo-1-pyrrolidinyl)oxy]carbonyl]phenyl]xanthylium, inner salt
Molecular Weight	623.51
Chemical structure	
Molecular Formula	$C_{31}H_{21}F_4N_3O_7$
PubChem identifier	134160239
SMILES	O=C(ON1C(CCC1=O)=O)C2=CC=C(C([O-])=O)C(C(C3=CC=C(N4CC(F)(F)C4)C=C3O5)=C(C=C/6)C5=CC6=[N+]7CC(F)(F)C\7)=C2
Source	Synthetic
InChiKey	VPHPPEABRFKVDC-UHFFFAOYSA-N
Licensing details	Sold under license from the Howard Hughes Medical Institute, Janelia Research Campus

References

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

Grimm JB et al (2017) Nature methods 14

PubMedID 28869757