BP Fluor 488 Antibody Labeling Kit

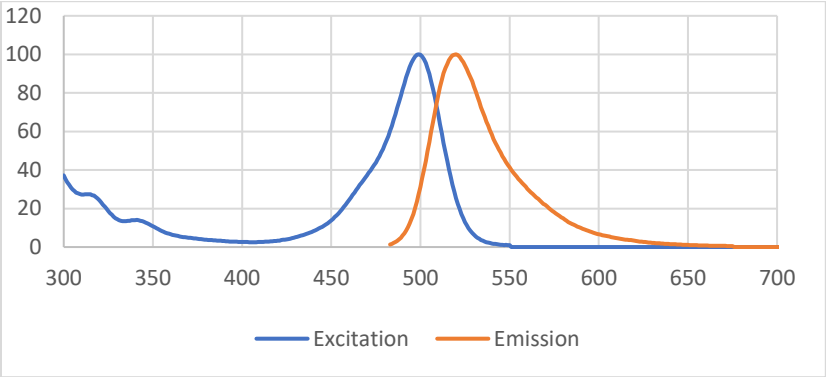
Components

Kit Component		Product size						storage
		BP Fluor 488 NHS antibody labeling kit			BP Fluor 488 Mal antibody labeling kit			
		BP-50017 (1x100 ug)	BP-50016 (3x100 ug)	BP-50015 (1x1 mg)	BP-50038 (1x100 ug)	BP-50037 (3x100 ug)	BP-50036 (1x1 mg)	
A	Active dye	1	3	1	1	3	1	-20C
B	Reaction Buffer	1	1	1	1	1	1	4-8C
C	Desalt column	1	3	1	1	3	1	4-8C
D	DMSO, 1 ml	1	1	1	1	1	1	4-8C
E	NaN_3 3% 0.5 ml	1	1	1	1	1	1	4-8C
Note BP Fluor 488 conjugation kit is available in two formats, NHS and maleimide. Other formats are available upon request.								

Overview

BP Fluor 488 (Alexa Fluor® 488 equivalent) is a green fluorescent dye for the 488 nm argon laser line. However, it carries multiple negative charges, which can significantly reduce conjugates aggregation in the bio application.
BP Fluor 488 (A_{max} 493 nm, E_{max} 519 nm) has absorption and emission spectra comparable to Fluorescein (FITC), but are much brighter, far more photostable and less sensitive to pH changes between pH 4 and 10. BP Fluor 488 conjugates are compatible with common fluorescein equipment, settings, or filters and are ideally suitable for all applications in fluorescence microscopy and flow cytometry with high requirements in sensitivity. Alexa Fluor 488 shows more persistent and brighter fluorescence than Fluorescein in epifluorescence microscopes even without the addition of anti-fading agents to aqueous mounting media. In permanent organic mounting media BP Fluor 488 is also more fluorescent and long-term stable than FITC.

	FITC	BP Fluor 488	Benefits
Spectrum	Abs./Emi. 495/519	495/519	Comparable spectrum, No need change settings or filter
Photostability	**	*****	high photostability, more time for observation and image capture.
Brightness	***	*****	Brighter, detect low-abundance protein helpful



Protocol

Please follow general protocol
---dye NHS antibody labeling kit
---dye maleimide antibody labeling kit

Application

FC, ICFC, IHC-F, IHC-P, ICC, IF,3-DIHC, IHC, WB,

FAQ

Q: My antibody has histidine and glycine in it, will this be a problem?
A: Yes. The reactive dye in the kit reacts with primary amine groups on histidine and glycine. This can result in less labeling of the antibody. The histidine and glycine must be removed, such as with dialysis or a desalting column. Other components that can interfere include BSA, gelatin, and Tris buffers.

Q: What formulation of antibody should I use for conjugation for small animal in vivo imaging?
A: Normally, antibodies should be in PBS buffer at a concentration of 0.5-3.0 mg/ml based on good reaction kinetics. The antibody must be free of preservatives (azide etc.), amine containing buffers and carrier proteins such as BSA.

Q: What is degree of labeling (DOL)?
A: Degree of labeling (DOL) is the number of fluorophores per antibody. For in vivo labeling experiments, the DOL is restricted to a narrow range because it has significant consequences for the biodistribution and clearance of the probe. For example, we have determined that the DOL range for BP Fluor 488 is 4 to 9 molecules per antibody for most of applications.

Q: Is BP Fluor 488 compatible with common fixatives, buffers, permeabilizing agents like formaldehyde, formalin, paraffin, xylene, Triton® X-100, etc.?

A: BP Fluor 488 dyes are compatible with Triton, formaldehyde, and formalin. Paraffin processing of tissues likely will extract dyes of almost any type, including BP Fluor 488 dyes, though they are fine to use after the processing, such as for immunohistochemistry (IHC) imaging.

Q: Is BP Fluor 488 dyes cell-permeant?
A: No, they are too highly charged.

Q what's the recommendation of BP Fluor 488 NHS: protein molar ratio (MR) and typical yield for labeling 12-150kDa protein?

Different proteins need different MR for labeling. User need optimize the ratio to get the appropriate ratio for their application accordingly. For overlabeling case, the fluorescence signal will be quenched and, in some cases, the conjugates will principate out. We highly recommend that you evaluate your protein conjugate in its intended application before you conclude that it is under or over labeled. A number of conditions can cause under or over labeling.

Table 1. Recommended BP Fluor 488 dye NHS: protein molar ratios (MR) and typical yields for labeling 12–150 kDa proteins

Protein (MW in kDa)	For Lower DOL	For Optimal DOL	For Higher DOL%	Yield
parvalbumin (12)	≤2	5	≥8	60
soybean trypsin inhibitor (20)	≤15	19	≥25	60
ovalbumin (40)	≤40	60	≥70	60
streptavidin (53)	≤20	30	≥50	90
transferrin (80)	≤6	12	≥15	70
F(ab)2 (100)	≤20	30	≥40	90
IgG (150)	≤25	55	≥65	90