Flamma® Fluors PS Bead

Introduction

Flamma® Fluors PS Bead provided by BioActs, as the applied material for diagnostics and biochemistry, etc., can be used for the unique performance according to fluorescent marker and organism particle size after loading our already developed various bright fluorescent dyes on the surface or inside. To conduct applied tests in each different field as such, we are progressing custom labeling in consideration of fluorescent wavelength and Intensity desired by users and we modified the surface using carboxylic acid so that a covalent bond of the materials such as nucleic acid, antigen, peptide, antibody, etc. can be possible. This Technical note provides a variety of information with applied field and tables using PS Bead. Polystyrene bead size can be supplied within the range of 100nm – 20um. For any larger sizes, please consult with support@bioacts.com.

Table.1 BioActs Flamma® Fluors PS Bead Products Group

Functionalized fluorescent PS Bead							
Cat. #	**Product**	**Ex**	**Em**	**Dia.**	**Modification**	**Packing size**	**Solids**
PSC4001	Flamma® Blue PS Bead	352	408	0.2um	COOH	2, 5, 10 mL	2%
PSC4002	Flamma® Blue PS Bead	352	408	0.5um	COOH	2, 5, 10 mL	2%
PSC4003	Flamma® Blue PS Bead	352	408	1um	COOH	2, 5, 10 mL	2%
PSC5001	Flamma® Green PS Bead	496	516	0.2um	COOH	2, 5, 10 mL	2%
PSC5002	Flamma® Green PS Bead	496	516	0.5um	COOH	2, 5, 10 mL	2%
PSC5003	Flamma® Green PS Bead	496	516	1um	COOH	2, 5, 10 mL	2%
PSC6001	Flamma® Red PS Bead	564	589	0.2um	COOH	2, 5, 10 mL	2%
PSC6002	Flamma® Red PS Bead	564	589	0.5um	COOH	2, 5, 10 mL	2%
PSC6003	Flamma® Red PS Bead	564	589	1um	COOH	2, 5, 10 mL	2%
PSC7001	Flamma® Deep Red PS Bead	638	651	0.2um	COOH	2, 5, 10 mL	2%
PSC7002	Flamma® Deep Red PS Bead	638	651	0.5um	COOH	2, 5, 10 mL	2%
PSC7003	Flamma® Deep Red PS Bead	638	651	1um	COOH	2, 5, 10 mL	2%
Non-functionalized fluorescent PS Bead							
Cat. #	**Product**	**Ex**	**Em**	**Dia.**	**Modification**	**Packing size**	**Solids**
PSS4004	Flamma® Blue PS Bead Calibration	383	447	6um	-	10 mL	2%
PSS5004	Flamma® Green PS Bead Calibration	395	510	6um	-	10 mL	2%
PSS8004	Flamma® R-PE PS Bead Calibration	542	575	6um	-	10 mL	2%
PSS3004	Flamma® PerCP PS Bead Calibration	482	677	6um	-	10 mL	2%
PSS4005	Flamma® Blue PS Bead Calibration	383	447	15um	-	10 mL	2%
PSS5005	Flamma® Green PS Bead Calibration	395	510	15um	-	10 mL	2%
PSS8005	Flamma® R-PE PS Bead Calibration	542	575	15um	-	10 mL	2%
PSS3005	Flamma® PerCP PS Bead Calibration	482	677	15um	-	10 mL	2%

Fluorescent dye spectra

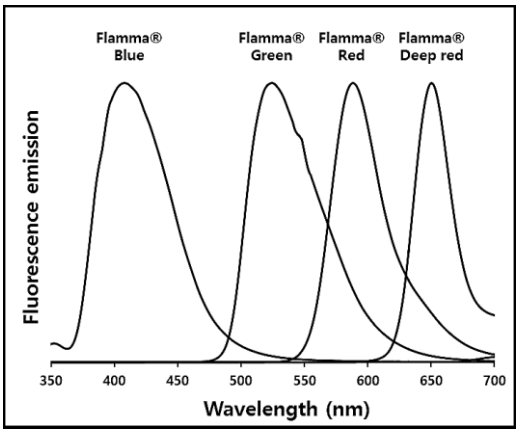


Figure 1. Information of dye introduced into PS Bead. From left, it shows fluorescence graph of Flamma® Blue, Flamma® Green, Flamma® Red, and Flamma® Deep red.

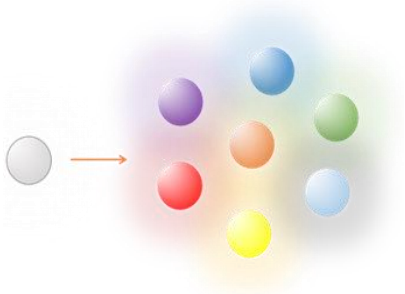
Excitation value and Emission value of each dye introduced into Flamma® Fluors PS Bead are stated in Figure 1. The stated spectra was analyzed by specific optical system of BioActs, which are supplied a constant spec through quality evaluation. We filled these fluorescent dyes into the bead using the special method developed by us so as to prevent photobleaching or quenching phenomenon that may appear in the specific environment

*Flamma® Blue : Excitation / Emission (352 / 408)
*Flamma® Green : Excitation / Emission (496 / 512)
*Flamma® Red : Excitation / Emission (564 / 589)
*Flamma® Deep Red : Excitation / Emission (638 / 651)

Handling & Storage condition

For all Flamma® Fluors PS Bead series, you can use stably in sonication and vortexing or shaking process. However, you need to avoid exposure to light in the storage process if possible. Temperature of 2 – 8 ℃ must be always kept and do not use the bead in a frozen condition. Recommended shelf life is one (1) year from the produced date and please be sure to check the expiry date. All the supplied Flamma® Fluors PS Beads are kept in water containing 0.05 % Sodium azide.

Custom labeling service



In the case of Bioimaging or Biosensing applied field, each different bead specification such as Bead size, Surface modification, Single or Multiple fluorescent dye, Fluorescence intensity is required depending on the user Bead size, Surface modification, Single or Multiple fluorescent dye, Fluorescence intensity, etc. Therefore, BioActs provides a customized service through feedback with the customer. Flamma® Fluors series suggested in Table 2 include full spectrum line from UV (Ultraviolet) to NIR (Near-infrared) category, which enables to provide fluorescent Spectrum bead in the filled condition as desired by the user. Flamma® Fluors series can adapt to all optical condition of fluorescent equipment. It is quite bright and clear and also can be used widely.

Table. 2 ㈜BioActs Flamma® Fluors series

Product	Abs_{Max} [1]	Em_{Max} [1]	Extinction [2]	CF_{280} [3]
Flamma® 406	401	434	26,000	0.70
Flamma® 496	496	520	82,000	0.19
Flamma® 552	550	565	150,000	0.07
Flamma® 560	554	627	110,000	0.46
Flamma® 648	648	663	250,000	0.03
Flamma® 675	675	691	220,000	0.09
Flamma® 749	749	774	220,000	0.03
Flamma® 774	774	800	200,000	0.10
Flamma® 800	775	795	240,000	0.03
FAM	494	518	70,000	0.20
TAMRA*	553	576	92,000	0.20
ICG*	785	812	240,000	0.05

Flamma® Fluors dyes and FAM were measured in PBS at pH 7.4. *TAMRA and ICG were measured in MeOH.
[1] Fluorescence Excitation and Emission maxima, in nm.
[2] Extinction coefficient at ε_{Max} in $cm^{-1}M^{-1}$.
[3] Correction Factor (A_{280} free dye / A_{Max} free dye).

Manual

Bead surface modification

An enormous quantity of Carboxylic acid exist on the surface of Functionalized PS bead. Such existence of Carboxylic acid on the PS bead surface enables to be combined with Carbodiimide compound, substituted to the effector such as N-Hydroxysuccinimide, and can be labelled to protein, antibody, etc. containing primary amines (-NH_2).

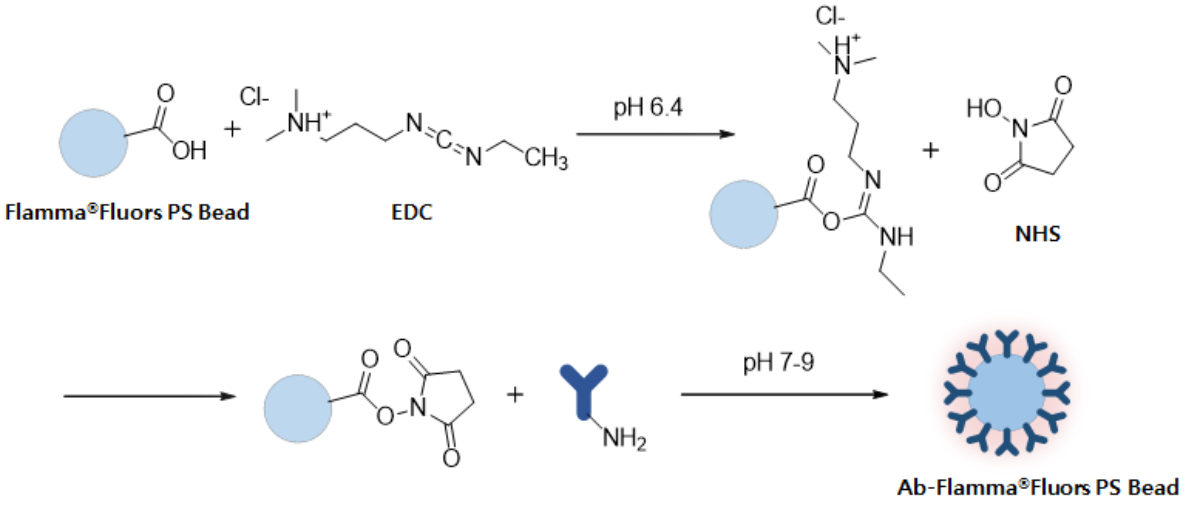


Figure 2. NHS ester reaction

Carboxylic acid activation

In order to check if Carboxylic acid on the surface of Flamma® Fluors PS Bead is active or not, we pre-treated it with EDC/NHS ester, changed the surface of PS Bead, treated the Amine fluorescent dye, and then analyzed its being active for Carboxylic acid (Figure 3).

Reagent to be prepared

- 50mM MES buffer (pH 6.4)
- 50mM Borate buffer (pH 8.5)
- N-Hydroxysuccinimide
- N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide
- Flamma® 552 amine (PWE1122)

Required equipment

- 1.5 mL microcentrifuge tube
- Micropipette
- centrifuge)
- incubator

Activation test

1. Move 20 uL of Flamma® Deep red (PSC7001) to 1.5 mL Tube.
2. Add 10uL of 50nM MES buffer, 10uL of N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide (10mg/mL), and 10uL of N-Hydroxysulfosuccinimide (10mg/mL), and then mix them for 10 minutes in the incubator.
3. Inject 245uL of 50mM Borate buffer and then stir them.
4. Melt up Flamma® 552 amine in Dimethylformamide at 2.1mg/mL.
5. Inject 5uL of 3. Sample solution into 4. Sample and then mix them in the incubator for 10 minutes.
6. Centrifuge (10,000rpm/10min) it and then separate 200uL of supernatant except the bead.
7. Put 2.8mL into 5mL tube and mix them to conduct analysis of absorbance value.

Activation result

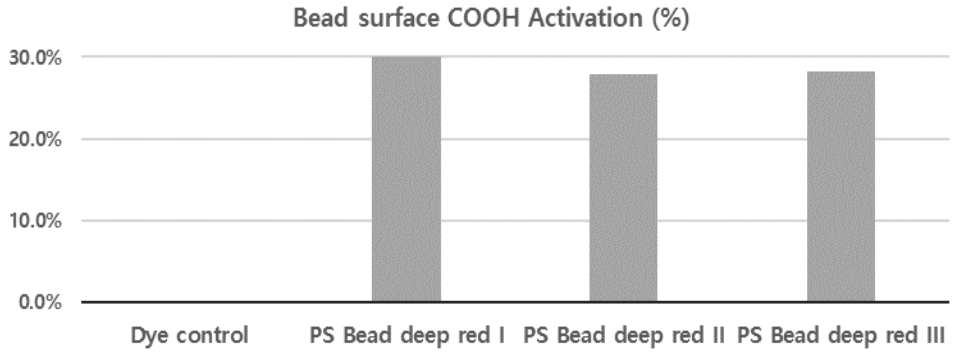


Figure 3. Bead surface COOH Activation test *Bead supernatant intensity: Attach Dye (552/565) after inducing NHS ester on the surface of Bead. Put down all the dye-attached beads and analyze the absorbance value of non-bound dye. The more the COOH of # Bead의 COOH, the less the Intensity of filtrate

Bead Surface COOH activation # Bead Activation % = (sample supernatant Abs intensity / control dye Abs intensity-100%)-1

Optical analysis

In the case of Flamma® Fluors PS Bead series, the dye is introduced into the PS Bead so as to minimize self-disturbance or mutual quenching of fluorescent Intensity. In addition, we confirmed fluorescent intensity being stable even after external stimulus of sonication, etc. Figure 4. is the photograph that compares intensity of fluorescence using fluorescence measuring equipment after sonication using Flamma® Deep red PS Bead.

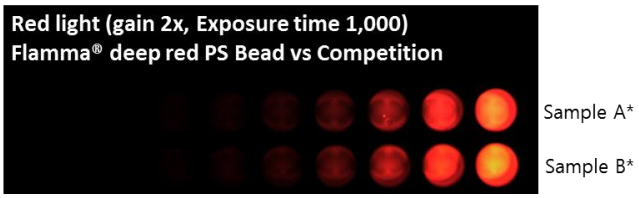


Figure 4. Flamma® Fluors PS Bead Optical analysis Sample A: Dark red PS Bead / Sample B: Flamma® Deep red PS Bead. In the case of optical analysis, comparative analysis was conducted by proceeding 0.2% from right and 1/2 serial dilution to left side.

Comparative analysis of the product's fluorescent intensity

1. Mix 20uL of 2% Fluorescent PS Bead with 180uL of D.W to produce the sample, final concentration of which is 0.2%.
2. Fill 100 uL of D.W each in the lane 1 to 7 on the upper lane of D.W 96 well black plate, while inject 200uL of the sample produced in para. In the lane 8.
3. Take 100 uL of the sample from the lane 8 and put it in the lane, and then conduct serial dilution from 7 to 2. (i.e. 1/2 serial dilution)
   Discard 100 uL of the final process of dilution so that the final volume of all wells can become 100 uL. (Lane 2)
   Make use of Lane 1 as a blank.
4. Compare fluorescent Intensity of PS bead by concentrations using a fluorometric analyzing equipment.

Coupling protocol

Reagent to be prepared

- Flamma® Deep red PS Bead (200nm)
- 50mM MES buffer (pH 6.4)
- 50mM Borate buffer (pH 8.5)
- N-Hydroxysuccinimide
- N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide
- Goat anti-mouse IgG
- BSA in PBS (10X)
- 1 M Glycine
- 1 X PB buffer (pH=7.4)

Required equipment

- 1.5 mL micro centrifuge tube
- Micropipette
- Centrifuge
- Incubator

#1. PS Bead surface activation

EDC/NHS coupling is one of the easiest and convenient skills of crosslinking technique of protein containing primary amine such as antibody. Primary amine existing in the protein such as antibody and lysine possesses in a physiologic pH, which is easy for conjugation as it exists outside the 3rd structure in the aqueous medium. Modify it with carboxy-NHS through the carboxylation-state EDC/NHS coupling of the surface of PS bead, and NHS terminus enables primary amine specific reaction among carboxyl group, primary group existing in the antibody.

1. Dilute 2% PS Bead by mixing 25 mM MES buffer (pH=5.0) into final 0.2% (pH=5.0).
2. After re-mixing N-Hydroxysuccinimide and N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide in the particle solution mixed in the para. 1, conduct a slow tilt rotation for 30 minutes at room temperature.
   # NHS ester / EDC (Final conc. 2mM) #Final volume: 500 uL.
3. Conduct centrifugation of 15,000Xg for 10minutes and then remove supernatant.
4. Re-float the bead pellet with 25 mM MES buffer (pH=5.0) and then repeat the para 3 process two times.

#2. Antibody coupling

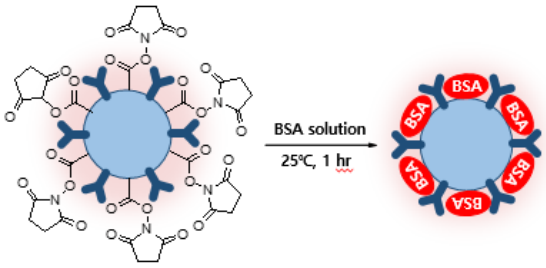
PS bead activated by NHS terminus comes to have reactivity to primary amine existing in the antibody, and finally antibody is conjugated on the surface of PS bead.

1. Dilute it by adding 30mM MES buffer (pH=6.0) to the bead pellet.
   #Final volume: 300uL
2. Add antibody solution to the bead suspension solution and let it react for 3-4 hr at room temperature.
   #Bead 1mg / Ab 100ug (Goat anti-mouse IgG)
3. Wash it three times with 0.01% Tween-20 (in PBS) solution.
   #Incubate it for 10 minutes at room temperature in the washing buffer in each washing process.

#3. Blocking

Nonspecific binding is the problem that should be looked after most carefully when you conduct a test using Flamma® PS Bead. Flamma® PS Bead has negative charge in general. In a biological

condition, most of nonspecific binding is caused by hydrophobic interaction. However, some charge-based interaction or nonspecific binding by crosslinker such as NHA may occur. Such problem can be solved by blocking the binding site that exists on the surface of particle using a large molecule such as BSA or polysaccharide.



1. Dilute 10X BSA (in PBS) solution into 1X, add antibody coupling PS bead, and then let it react for 30 min-2 hr at room temperature or 37℃.
2. Afterwards, wash it with DW.

#4. Quenching

In order to increase specificity, blocking of binding site using small molecule as well as blocking of binding site using large molecule is also necessary. By blocking using small molecule such as Glycine, binding of small-sized nonspecific ligand in addition to large-sized ligand can be prevented.

1. Add already prepared 1M Glycine so that the final concentration can be 50mM and then let it react for 1 hour at room temperature.

#5. Storage

1. Remove supernatant by separating the finally reacting solution at 15,000Xg for 10 minutes.
2. Float bead pellets in the finally stored solution (1X PB buffer) and keep it at 2-8 ℃.

References

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SDSs (Safety Data Sheets) can be found at formal BioActs website www.bioacts.com.

CoA (Certificate of Analysis) offers detailed information on quality of each product. To check CoA, confirm by checking lot no. marked on the cover of each product page of BioActs formal website www.bioacts.com. If confirmation is difficult, please contact our technique support part.

This product can be used only for research purpose and can't be used for treating or diagnosing of human organism or animal.

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