

# Protein A - HiLyte™ Fluor Conjugates

## → Benefits

### Universal

Only one reagent to detect multiple targets.

### Large panel of emission wavelengths

The emission fluorescence of the HiLyte™ Fluor spans the full visible and near infrared spectra.

### Super bright signal

HiLyte™ Fluor exhibit more intense fluorescence compared to classical dyes (TAMRA, FITC, ROX, etc).

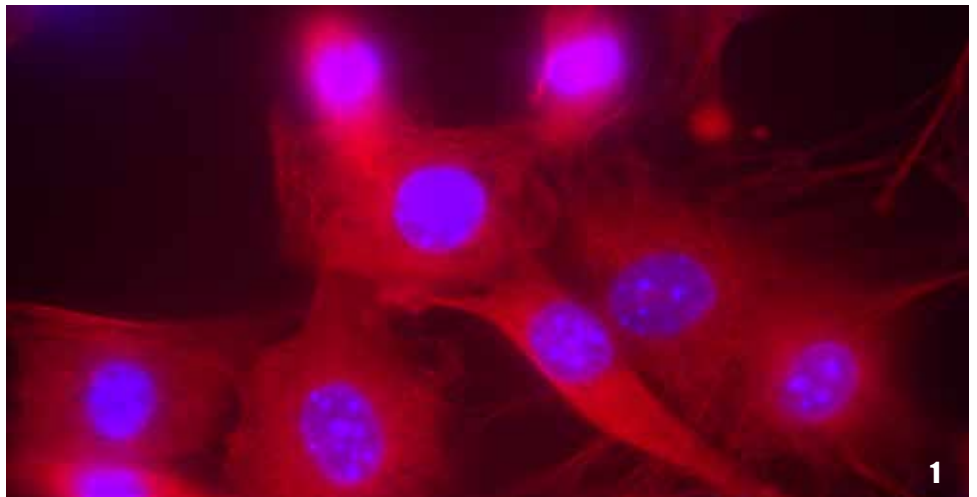
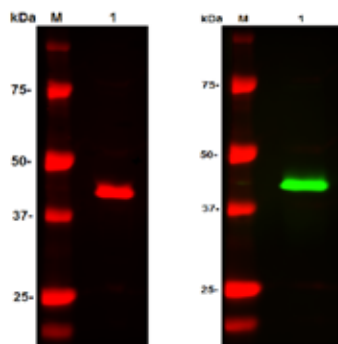
Protein A - HiLyte™ Fluor conjugates are excellent alternatives to dye-labeled secondary antibodies. These highly efficient conjugates constitute a series of universal detection reagents, which combine the binding capacity of Protein A with the high fluorescent intensity of HiLyte™ Fluor dyes.

Protein A-HiLyte™ Fluor conjugates provide a super bright signal. They can be used to detect primary antibodies in every protein detection application, e.g. Western blot (WB), Immunohistochemistry (IHC) and Immunofluorescence (IF).

## → Results

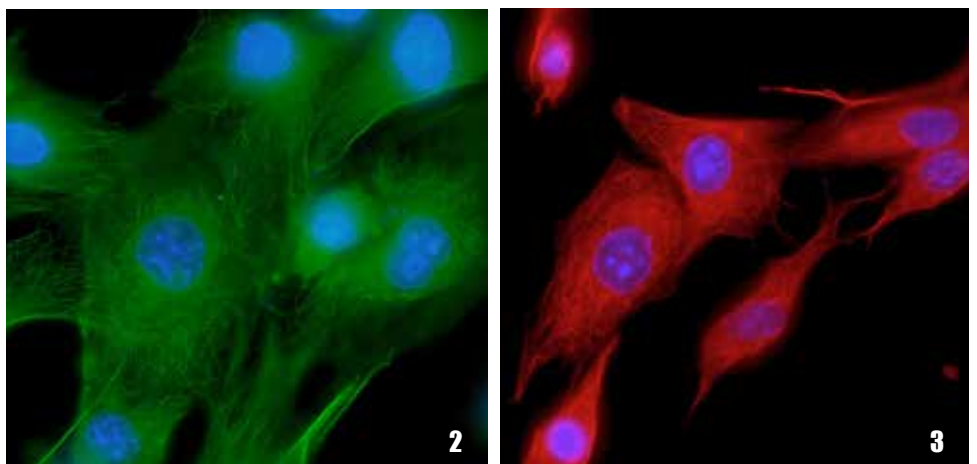
### Western Blot Results

3T3 Cell Lysate at 10 µL/lane was resolved on SDS-PAGE and transferred to nitrocellulose membrane. Membrane was probed with rabbit anti-Actin antibodies at 1 µg/mL (Cat.# AS-29553). Anti-Actin antibodies were detected with either Protein A-HiLyte™ Fluor 680 (left-Cat.# AS-72238) or Protein A-HiLyte™ Fluor 750 (right - Cat.#AS-72239) reagent at 0.2 µg/mL\*.



### IF Results

3T3 cells were stained with Rabbit-anti-tubulin IgG (0.5 µg/mL) followed by Protein A-HiLyte™ Fluor 488 (2), Protein A-HiLyte™ Fluor 555 (3) and Protein A-HiLyte™ Fluor 647 (1) at 0.5 µg/mL\*.



\*Please note that the Protein A-HiLyte™ Fluor Conjugate amount mentioned above is experiment dependent. Optimization may be required to obtain the best results.

## Protein A

Protein A is a non-glycosylated cell-wall protein from *Staphylococcus aureus*<sup>1-4</sup> containing five IgG binding domains (A,B,C,D and E). These domains have a strong affinity for the Fc part of immunoglobulins from different species. Highly purified recombinant Protein A was expressed in recombinant bacterial expression system. It consists of only the IgG binding domains, with a final purity level of >99%. The apparent molecular mass is approximately 30,000 compared to 42,000 for the native Protein A found in *Staphylococcus aureus*. Each domain is approximately 60 amino acids long with no cysteine present.<sup>3</sup>

### Recombinant Protein A affinity for Ig from various species

Species	Immunoglobulin	Protein A binding
Human	IgG1, IgG2, IgG4	Strong
	IgM, IgA	Weak
Mouse	IgG1	Weak
	IgG2A, IgG2B, IgG3	Strong
	IgM	None
Rat	IgG1	Weak
	IgG2C	Strong
Goat	IgG1	Weak
	IgG2	Strong
Bovine	IgG2	Strong
Guinea Pig	IgG	Strong
Rabbit	IgG	Strong

### Catalog Numbers

Product Name	Quantity	Catalog#
Protein A-HiLyte™ FLuor 488 Conjugate	1mg	AS-72235
Protein A-HiLyte™ FLuor 555 Conjugate	1mg	AS-72236
Protein A-HiLyte™ FLuor 647 Conjugate	1mg	AS-72237
Protein A-HiLyte™ FLuor 680 Conjugate	1mg	AS-72238
Protein A-HiLyte™ FLuor 750 Conjugate	1 mg	AS-72239

### Related Product

Unlabeled recombinant Protein A and biotin conjugated Protein A are also available. Unlabeled Protein A can be used for immunoprecipitation, antibodies purification and assay development.

Product Name	Quantity	Catalog#
Protein A, recombinant	5 mg	AS-72233-5
	25 mg	AS-72233-25
	100 mg	AS-72233-100
	1g	AS-72233-1000
Protein A-Biotin Conjugate	5 mg	AS-72234

HiLyte™ Fluor is a trademark of AnaSpec, Inc.

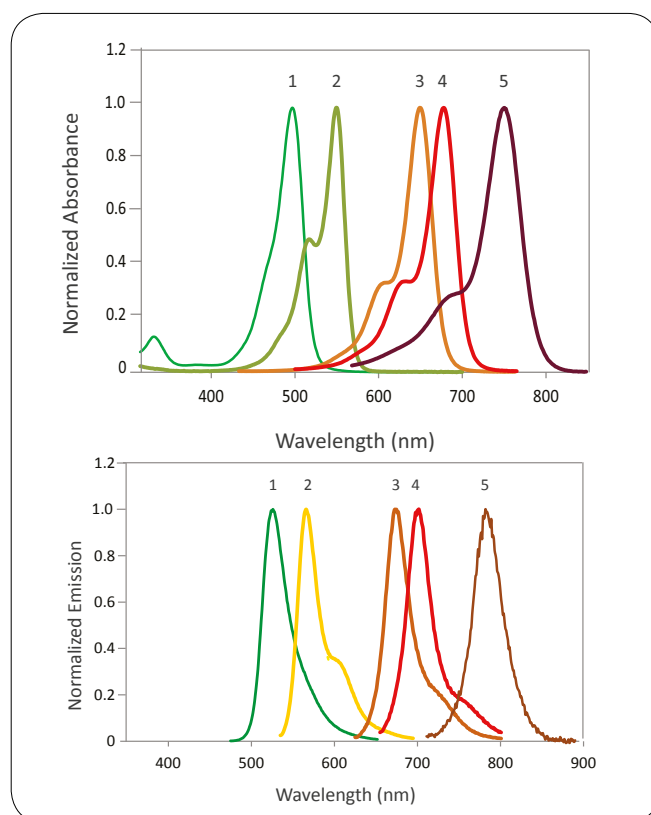
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#### References

1. Björk, I. Eur J Biochem 29 (1972): 579-584.
2. Sjödaahl, J. Eur J Biochem 78 (1977): 471-490.
3. Schröder, A. K. et al. Immunol 57 (1985): 305-309.
4. Rosenburg, I.M. Protein Analysis and Purification, Birkhäuser, 2005: 41-47.

## HiLyte™ Fluor Dyes

HiLyte™ Fluor Dyes are AnaSpec, EGT Group's proprietary Fluorescent Dyes. They are pH insensitive, highly photostable and brighter than the classical fluorescent dyes. They span the full visible spectrum and can be used for multiple applications.



Dye	Ex/Em (nm/nm)
HiLyte™ Fluor 488	499/523
HiLyte™ Fluor 555	553/568
HiLyte™ Fluor 647	649/674
HiLyte™ Fluor 680	678/699
HiLyte™ Fluor 750	754/778

Normalized absorbance spectra (graph↑) and normalized fluorescence spectra (graph↓) of HiLyte™ Fluor 488 (1), HiLyte™ Fluor 555 (2), HiLyte™ Fluor 647 (3), HiLyte™ Fluor 680 (4), and HiLyte™ Fluor 750 (5).



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