コスモ・バイオ株式会社

JNC CORPORATION

Biotin-Aequorin : Biotin Labeled Ca^{2+}-Binding Photoprotein

Cat. No.:	T-003
Product Code:	BS-AQ
Source:	Recombinant protein expressed in *E. coli*.
Form:	Liquid
Constituents:	50 mM Tris-HCl (pH 7.6)－10 mM EDTA－1.2 M $(NH_4)_2SO_4$
Purity:	> 95% (SDS-PAGE under reducing conditions)
Preservative:	None
Recommended Storage:	Store at -80 °C upon receipt.
Shipping condition:	Shipping with dry ice.
Size:	50 µg
Protein concentrations:	1 mg/mL (OD_{280} = 2.97 in 0.1 % solution)
Remarks:	Avoid contamination of Ca^{2+}.
Uses:	Highly sensitive immunoassay through avidin (streptavidin)-biotin complex (ABC).

References:

1) Inouye, S. and Sato, J. (2008) Recombinant aequorin with a reactive cysteine residue for conjugation with maleimide-activated antibody. *Anal. Biochem.* 378: 105-107.
2) Inouye, S. and Sato, J. (2008) Comparison of luminescent immunoassay using biotinylated proteins of aequorin, alkaline phosphatase and horseradish peroxidase as a reporter. *Biosci. Biotechnol. Biochem.* 72: 3310-3313.

Laboratory Reagent For Research Use Only

Supplier

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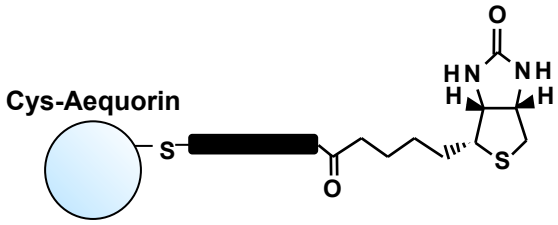
Biotin labeled Ca^{2+}-binding photoprotein

Biotin-Aequorin

Product code : BS-AQ

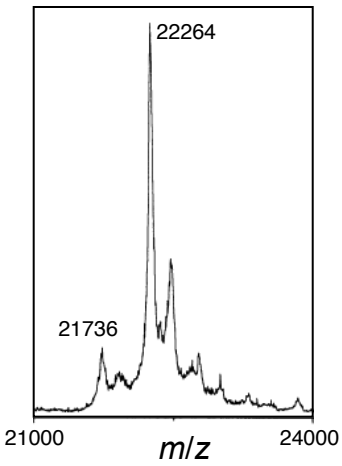
- **Biotinylated Cys-Aequorin.**
- **The conjugation ratio of aequorin to biotin is 1 : 1.**

Biotin-Aequorin



	Cal. Mass
Cys-Aequorin	21,735.3
Biotin-Aequorin	22,261.0

Spectrum of MALDI-TOF-MS



The feature of high sensitive aequorin assay

✓ **Ca^{2+} specific reaction**
No background. No false positive.
It is not necessary to prepare a reagent for detection.

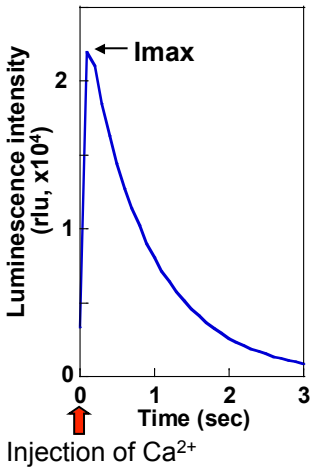
✓ **Flash luminescence**
High signal to noise ratio (high S/N ratio).
Less time to acquire results.

✓ **High sensitivity**
> 3 fg per assay, 100 times higher sensitivity than firefly luciferase.

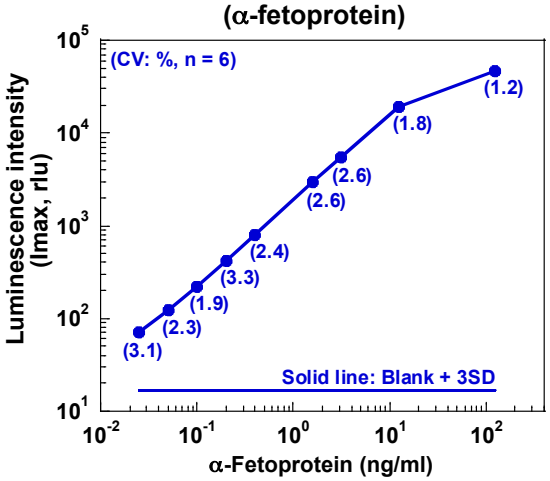
Immunoassay using biotin-aequorin

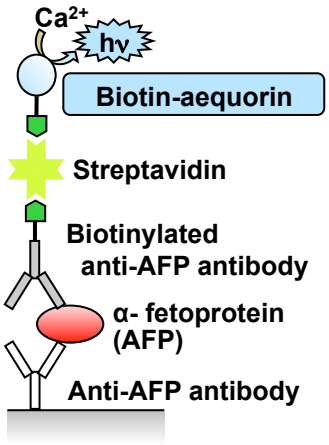
Detection of aequorin flash luminescence is performed only by the adding of Ca^{2+}, without a substrate.

Luminescence pattern



Standard curve of tumor marker (α-fetoprotein)





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Biotin-Aequorin

Product Code. BS-AQ
Lot. No. 808-HS-D
50 μg (1 mg/mL in 50 mM Tris-HCl (pH 7.6), 10 mM EDTA containing 1.2M $(NH_4)_2SO_4$)
Store at –80℃.

Introduction

Aequorin is a Ca^{2+}-binding photoprotein found in the luminous jellyfish *Aequorea*. Aequorin is made up of apoaequorin and 2-peroxycoelenterazine. Aequorin emits blue light (λ_{max} = 460 nm) by an intramolecular reaction upon reacting with Ca^{2+}, and decomposes into apoaequorin, coelenteramide and CO_2. Recombinant aequorin is prepared from apoaequorin expressed in *E.coli* cells with coelenterazine.[1) ~ 5)]

Biotin-Aequorin is prepared from Cys-aequorin[6)] with maleimide-activated biotin. Cys-aequorin is a mutated recombinant aequorin possessing a reactive –SH group for chemical conjugation. The conjugation ratio of aequorin to biotin is 1 : 1. Biotin-Aequorin enables various highly sensitive assays through avidin (streptavidin)-biotin complex[7)] (ABC).

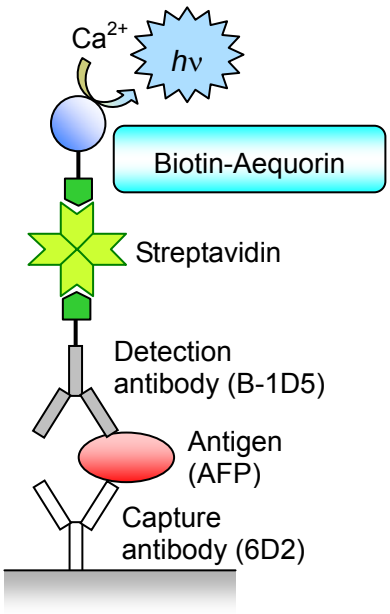
Important product information

- Purity: >95% on SDS-PAGE analysis by the Laemmli method.
- Concentration of aequorin: The concentration of recombinant aequorin was determined by the absorbance value of 2.97 in 0.1 % solution at 280 nm.
- Storage/handling: Avoid contamination with Ca^{2+} because aequorin is Ca^{2+} sensitive (>10^{-7} M). All solutions of aequorin should be free of Ca^{2+} (For example, add EDTA to all solutions).
- For use, keep aequorin at 4 ~ 10 ℃. And then, store at -80℃, immediately.
- Addition of 0.1 % BSA to buffer will help increase stability at room temperature.
- Only use for biochemical research. Don't use this product for medical or pharmaceutical purposes (For example, medical treatment or clinical diagnosis for humans and animals).

Example procedure for Immunoassay using Biotin-Aequorin in 96 well plate

Reagents

1. **Capture antibody**
   Anti-α fetoprotein (6D2; 5 μg/ml)
2. **Detection antibody**
   Biotinylated anti-α fetoprotein (B-1D5; 74.9 ng/ml = 497 fmol/ml)
3. **Antigen**
   Human α-fetoprotein standard (AFP; 0.0025 ~ 125 ng/ml)
4. **Streptavidin**
   Streptavidin (SA; 6 μg/ml = 100 pmol/ml)
5. **Biotin-Aequorin**
   Biotin-Aequorin (B-AQ; 2.2 μg/ml = 100 pmol/ml)
6. **Coating buffer**
   50 mM Carbonate buffer (pH 9.6)
7. **TBS**
   20 mM Tris-HCl (pH 7.6) and 150 mM NaCl
8. **Blocking buffer**
   1 % bovine serum albumin, 2 mM EDTA and 0.05 % NaN_3 in TBS
9. **Washing buffer**
   0.05 % Tween 20 and 2 mM EDTA in TBS
10. **Antigen diluent buffer**
    10 % Block Ace and 0.05 % Tween 20 in TBS
11. **Aequorin diluent buffer**
    10 % Block Ace and 5 mM EDTA in TBS
12. **$CaCl_2$ solution**
    50 mM $CaCl_2$ in 50 mM Tris-HCl (pH 7.6)



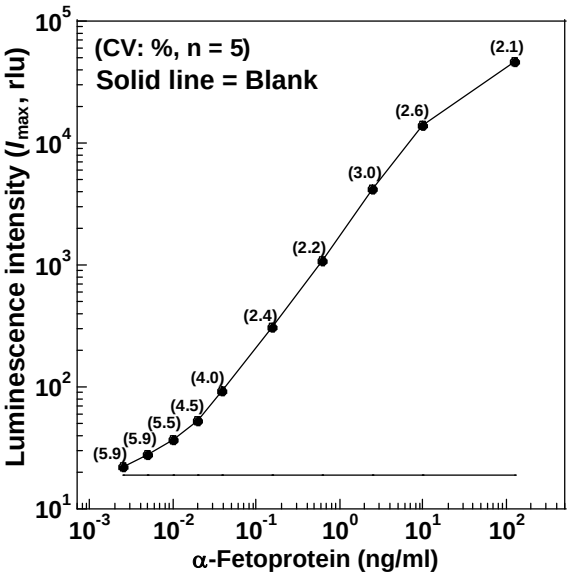
Procedure

1. **Coating of capture antibody**
   Coat microwells with 100 μl/well of capture antibody (6D2) diluted in Coating buffer.
   Incubate overnight at 30℃.
2. **Washing**
   Aspirate and wash 3 times with >300 μl/well of Washing buffer.
3. **Blocking**
   Block plate with 200 μl/well of Blocking buffer. Incubate overnight at 4℃.
   Aspirate and wash 3 times as in step 2.
4. **Reaction of antigen**
   Add 100 μl/well of antigen (AFP) diluted in Antigen diluent buffer. Incubate 1 hr at 30℃.
   Aspirate and wash 3 times as in step 2.
5. **Reaction of detection antibody**
   Add 100 μl/well of detection antibody (B-1D5) diluted in antigen diluent buffer. Incubate 1 hr at 30℃.
   Aspirate and wash 3 times as in step 2.
6. **Preparation of complex of streptavidin and biotin-aequorin**
   Mix each 50 μl SA and B-AQ diluted in aequorin diluent buffer. Incubate 30 min at 30℃(ABC solution, **molar ratio; SA : B-AQ = 1 : 1**).
   Dilute ABC solution to 80 times with aequorin diluent.
7. **Reaction of ABC solution**
   Aspirate B-1D5 and wash 3 times as in step 2.
   Add 100 μl per well of diluted ABC solution. Incubate 30 min at 30℃.
   Aspirate and wash 3 times as in step 2.
8. **Adding Ca^{2+} solution and measure luminescence intensity**
   Inject 100 μl Ca^{2+} solution into the wells and measure maximum luminescence intensity (I_{max}) in 0.1 sec interval for 5 sec.

Results

AFP (ng/ml)	I_{max} (rlu)	SD	CV (%)	S/N ratio (Signal/Blank)
0	15	1	8.7	0.8
0.0025	22	1	5.9	1.1
0.005	28	2	5.9	1.4
0.01	37	2	5.5	1.9
0.02	53	2	4.5	2.7
0.039	93	4	4.0	4.8
0.156	311	7	2.4	16.0
0.625	1071	24	2.2	55.1
2.5	4159	123	3.0	214.1
10	13840	354	2.6	712.5
125	46926	981	2.1	2415.8

n = 5
Blank = 19: (Imax + 3 x SD) of 0 ng/ml AFP



References

1. Inouye, S., Noguchi, M., Sakaki, Y., Takagi, Y., Miyata, T., Iwanaga, S., Miyata, T. and Tsuji, F.I. (1985) Cloning and sequence analysis of cDNA for the luminescent protein aequorin. *Proc. Natl. Acad. Sci. USA,* 82: 3154-3158.
2. Inouye, S., Aoyama, S., Miyata, T., Tsuji, F.I. and Sakaki, Y. (1989) Overexpression and purification of the recombinant Ca^{2+}-binding protein, apoaequorin. *J. Biochem*. 105: 473-477.
3. Inouye, S., Zenno, S., Sakaki, Y. and Tsuji, F.I. (1991) High-level expression and purification of apoaequorin. *Protein Expr. and Purif.* 2: 122-126.
4. Shimomura, O. and Inouye, S. (1999) The *in situ* regeneration and extraction of recombinant aequorin from *Escherichia coli* cells and the purification of extracted aequorin. *Protein Expr. and Purif*. 16:

91-95.

5. Head, J.F., Inouye, S., Teranishi, K. and Shimomura, O. (2000) The crystal structure of the photoprotein aequorin at 2,3 A resolution. *Nature*, 405: 372-376.
6. Inouye, S. and Sato, J. (2008) Recombinant aequorin with a reactive cysteine residue for conjugation with maleimide-activated antibody. *Anal. Biochem.* 378: 105-107
7. Inouye, S. and Sato, J. (2008) Comparison of luminescent immunoassay using Biotinylated proteins of aequorin, alkaline phosphatase and horseradish peroxidase as a reporter. *Biosci. Biotechnol. Biochem.* 72: 3310-3313.

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