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

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

#### References:

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**Laboratory Reagent For Research Use Only**

Not for resale without prior written consent from JNC Corporation.
Biotin-Aequorin

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

**Spectrum of MALDI-TOF-MS**

<table>
<thead>
<tr>
<th>Cal. Mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cys-Aequorin</td>
</tr>
<tr>
<td>Biotin-Aequorin</td>
</tr>
</tbody>
</table>

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.

**Imunoassay using biotin-aequorin**

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

**Injection of Ca$^{2+}$**

**Standard curve of tumor marker ($\alpha$-fetoprotein)**

**Contact**

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**Product code**: BS-AQ

**Cat. No.**: T-003
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 (NH₄)₂SO₄)
Store at –80°C.

Introduction

Aequorin is a Ca²⁺-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 Ca²⁺, and decomposes into apoaequorin, coelenteramide and CO₂. Recombinant aequorin is prepared from apoaequorin expressed in E.coli cells with coelenterazine.1) ~ 5)

Biotin-Aequorin is prepared from Cys-aequorin 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 (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 Ca²⁺ because aequorin is Ca²⁺ sensitive (>10⁻⁷ M). All solutions of aequorin should be free of Ca²⁺ (For example, add EDTA to all solutions).
- For use, keep aequorin at 4 ~ 10 °C. And then, store at -80°C, 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 % NaN₃ 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. CaCl₂ solution
    50 mM CaCl₂ 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°C.

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°C.
   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°C.
   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°C.
   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°C (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°C.
   Aspirate and wash 3 times as in step 2.

8. Adding Ca²⁺ solution and measure luminescence intensity
   Inject 100 µl Ca²⁺ solution into the wells and measure maximum luminescence intensity (I_max) in 0.1 sec interval for 5 sec.

Results

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

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

References

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:
