

Safety warnings and precautions

Correct operations are necessary Safety warnings and precautions of this product. The complete instructions should be read and fully understood before attempting to use the product.

The Procedure described in the instruction manual applies only to the use for the intended purpose. Using the product for any purpose other than the intended use or in any manner other than that described in the manual is forbidden.

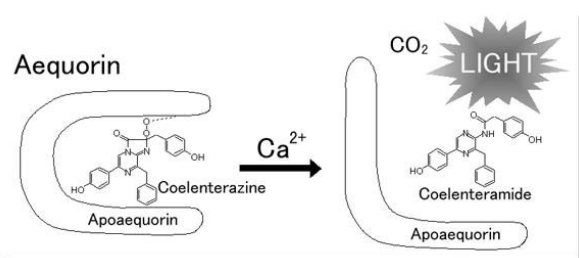
User shall be liable for all safety measures needed for any use other than specified in the manual.

Introduction

Aequorin is a photoprotein and a useful tool in biochemical and molecular biology for the measurement of calcium levels (concentration).

Components

Aequorin 50µg / bottle



Aequorin is the complex of the apoprotein apoaequorin and the prosthetic group coelenterazine, a luciferin whose wavelength is 470nm.

Aequorin can be used for detection of intracellular calcium ion concentration changes.

Specification

Model	AB-2900 Aequorin
Material	Recombinant Aequorin
Purity	More than 95%(verified by CBB staining after SDS-PAGE)
Stock solution	10 mM Tris-HCl(pH7.5), 2mM EDTA, 1.2M (NH ₄) ₂ SO ₄

Storage

The reagent should be stored at -80°C, only in case of short term using(within 1week) it can be stored at -20°C.

Do not store it after dilution, since it may lose its activity.

Disposal

Follow the procedure specified at your laboratory.

- Container Lid : Polypropylene
- Tube : Polypropylene
- O ring : Ethylene / Polyethylene

Additional equipments

Live-cell imaging	AB-3000B Cellgraph
Multi-plate luminometer	AB-2350 Phelios
Single-tube luminometer	AB-2270 OCTA

Precautions for Use

Please read following notes and fully understand it prior to use, since it required knowledge and experience in the reagents features and characteristics.

1. Aequorin is for research use only. Do not use this product for any other purpose.
2. Consider to prevent to be mixed with calcium ion when it is out of experiment, otherwise it proceed to react by meeting with calcium ion.
3. When store it for long term, it should be in $(\text{NH}_4)_2\text{SO}_4$ solution kept more than 1.2M at -80°C .
4. In emergency case, such as when it enters into eyes, or contacts with skin, or airborne droplet enters into a mouth, wash with flush water and see a doctor immediately.

Procedure

As aequorin is not permeable through plasma-membrane, so that it microinjects into the cell or the other methods which accelerate (enable) plasma-membrane permeability temporarily.

Once the intracellular calcium ion concentration rises, Aequorin binds to calcium and emits the light.

The intracellular aequorin can be used for observation of calcium ion concentration changes in long term, since it distributes in cytoplasm uniformly without going out to extracellular compartment or entering into subcellular organelle or small alveolus.

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.J. and Sakaki, Y. (1989) Overexpression and purification of the recombinant Ca^{2+} -binding protein, apoequorin. *J. Biochem.* 105:473-477.
3. Inouye, S., Zenno, S., Sakaki, Y. and Tsuji, F.I. (1991) High-level production and purification of apoequorin. *Protein Expression and Purification* 2: 122-126
4. Shimomura, O. and Inouye, S. (1991) The in situ regeneration and extraction of recombinant aequorin from *Escherichia coli* cells and the purification of extracted aequorin. *Protein Expression and Purification.* 16:91-95



ATTO CORPORATION
3-2-2 Motoasakusa Tel +81 3 5827 4863
Taito-ku Fax +81 3 5827 6647
Tokyo 111-0041 E-mail: eig@atto.co.jp
Japan http://www.atto.co.jp

Distributed by: