Biotin Labeling Kit for Avi-tag Protein (BirA)

Product Description:

The Avi tag is a short peptide tag composed of 15 amino acids, and BirA is able to link biotin to the lysine residue of the Avi tag in the presence of ATP and biotin to achieve biotin labeling of the protein of interest. This method has several advantages, including small Avi tag and low impact on fusion proteins, high biotin labeling efficiency and good reproducibility, and high affinity of the labeled protein to Streptavidin.

After AVI tag protein biotin labeling, it can be applied to a variety of biological detection methods, such as immunofluorescence, in situ hybridization, or flow cytometry, for visualization and quantification. In addition, the binding system of biotin and streptavidin has the advantages of high affinity, high sensitivity, strong specificity and good stability, and is widely used in a variety of detection systems.

The Biotin Labeling Kit for Avi-tag Protein (BirA) is a tool for biotin labeling of proteins or peptides fused with Avi tags using biotin ligase (BirA). This kit enables efficient and fast biotin labeling, which can be used to detect and purify target proteins using Streptavidin or Avidin.

Composition and storage conditions

Size / Components	20 Assays	100 Assays	Storage
BirA (100X)	10 μL	50 μL	-20℃
Biotin Ligase Buffer A (10X)	100 μL	0.5 mL	-20℃
Biotin Ligase Buffer B (10X)	100 μL	0.5 mL	-20℃
Shipping: Blue ice		**Shelf life: 12 months**	

Experimental manipulation

1. In vitro biotin labeling proteins or peptides tagged with Avi.

Prepare a reaction system according to the table below. After gently pipetting and mixing, incubate at 30°C for 30 minutes to complete biotin labeling of proteins or peptides tagged with Avi.

Components	Volume	Final Concentration

Biotin Ligase Buffer A (10X)	5 μL	1X
Biotin Ligase Buffer B (10X)	5 μL	1X
BirA (100X)	0.5 μL	1X
Protein or peptide	X μL	40 μM
ddH_2O	To 50 μL	-

***Note:**

1: The volume of this reaction system can be reduced or enlarged according to actual needs. In many cases, it can also be set to a reaction system of 20 μL.

2: The final concentration of protein (peptide) substrate should generally not exceed 40 μM.

3: If the final concentration of the protein (peptide) substrate is less than 40 μM, in order to ensure the biotin labeling effect, it is necessary to extend the reaction time appropriately, or increase the amount of BirA, or concentrate the protein (peptide) substrate to increase the concentration. For example, a final concentration of 20 μM in a protein (peptide) substrate can extend the reaction time to 1 hour; If you need to make the final concentration of 20 μM protein (peptide) substrate almost completely labeled with biotin in 30 minutes, you need to add about 2 times the amount of BirA.

4: If the protein (peptide) substrate must be low temperature to maintain activity, the reaction system can be placed at 4°C, the reaction time can be appropriately extended, and the amount of BirA can be appropriately increased.

2. Biotin labeling efficiency assay.

Detection can be performed by electrophoresis and blot detection experiments similar to Western blot, i.e., washing after electrophoresis, transfer, blocking, followed by incubation with appropriately diluted horseradish peroxidase-labeled Streptavidin, 3-5 washes, and then detection of biotin labeling efficiency by chemiluminescence imaging using ECL-like reagents.

3. Biotinylated protein or peptide purification.

Streptavidin agarose or magnetic beads are recommended for the separation and purification of biotinylated proteins or peptides.

Precautions

1. Care should be taken to avoid repeated freeze-thaw cycles of Biotin Ligase Buffer B (10X) to maintain its viability, and aliquots are recommended after the first thaw.
2. The dosage, reaction temperature, and time of BirA can be optimized according to the experimental needs, e.g. the concentration of BirA can be adjusted between 0.2X and 2X.
3. .The protein (peptide) substrate of this product is an Avi-tagged fusion protein or peptide.
4. There are certain requirements for the final concentration of salts or reagents in the reaction solution, such as NaCl < 100 mM, Glycerol < 5%, $(NH_4)_2SO_4$ < 50 mM, Tris < 50 mM, otherwise the activity of biotin ligase will be inhibited.
5. This product is for scientific research purposes only.

APExBIO Technology
www.apexbt.com
7505 Fannin street, Suite 410, Houston, TX 77054.
Tel: +1-832-696-8203 | Fax: +1-832-641-3177 | Email: info@apexbt.com