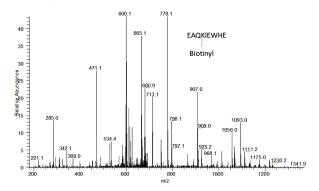
Biotin ligase BirA

Cat.# PT-EZ-BirA-100 100 ug **Cat.# PT-EZ-BirA-1000** 1000 ug

Description: Biotin ligase (BirA) ligates biotin specifically to a biotin acceptor peptide (**GLNDIFEAQ**<u>K</u>**IEWHE**). This product comes with reaction mixture (10X concentration: 0.5M bicine buffer, pH 8.3, 100mM ATP, 100mM MgCl₂, 500μM d-biotin)

Quality control assay: Biotin acceptor peptide tag fusion protein was used as a substrate. BirA activity was verified by streptavidin binding and mass spec analysis. A >95% bitinylation of the substrate was confirmed under the experimental conditions.



Storage conditions: Long term storage at – 80 C. Avoid repeated freeze-thaw cycles.

Purity: >80% by SDS-PAGE gel

Concentration: 0.5 mg/mL

Formulation: 20 mM MES buffer at pH5.5, 50 mM NaCl, 10% glycerol.

Reference:

1. Chen, I., Howarth, M., Lin, W., & Ting, A. Y. (2005). Site-specific labeling of cell surface proteins with biophysical probes using biotin ligase. *Nature methods*, *2*(2), 99-104.

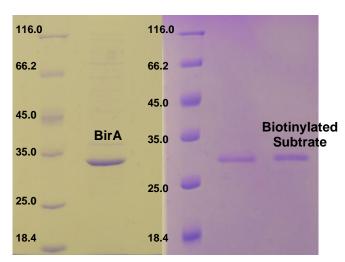


Figure 1. BirA SDS PAGE gel

Lane 1. Marker

Lane 2. BirA 1ug

Lane 3. Marker

Lane 4. Biotin acceptor substrate

Lane 5. Biotinylated substrate

Protocol for biotinylation

- 1. BirA reaction condition: 50mM bicine buffer, pH 8.3, 10mM ATP, 10mM MgCl₂, $50\mu M$ d-biotin.
- 2. Substrate concentration should be around 0.5-1mg/mL. Add 10-50 ug birA per mL reaction volume.
- 3. Incubate at room temperature for 1 hour or more. Longer reaction time is recommended at lower temperature.

Note:

- Biotin/biotinylated proteins are more soluble in bicine buffer, therefore it is recommended.
- 2. High glycerol and NaCl concentration may lower birA acitivity