FT-UP99476

FeBABE

A unique tool to study the three dimensional structure of protein complexes

Specifications

Part number :	UP994760		
Nom(s) :	p-Bromoacetamidobenzyl-EDTA, iron (III) chelate, MW: 589.14 (S)-[1-((bis(carboxymethyl)amino)methyl))-2-(4-(2-bromoacetyl)amino)phenylethyl)(carboxymethyl)amino)-acetic acid, iron(III), monohydrate		
Packaging :	1mg	Appearance:	yellowish brown powder
Storage :	–20°C	Purity:	> 95.0% (HPLC)
Shipping :	Room Temperature	Solubility:	1 mg/ml in H_2O, >20mM in DMSO
		Water content:	3.0-6.0 %

Features:

- mild conjugation reaction with a peptide or a protein (to SH groups, and even NH2 in conjunction with use of 2-imminothilane)
- mild cleavage reaction condition (milder than bromoacetamide)
- rapid reaction and high yield
- chain cleavage reaction of nucleotides or proteins with no sequence specificity.

Applications

- Determination of protein 3-D structures
- Rapid site-specific peptide / DNA cleavage reaction
- Determination of spacial relationships within protein subunits
- Prepare metal-conjugated biomaterials (pharmaceutical and diagnostic probes with In3+-chelated EDTA)

General information

- BABE, a Meares' reagent, and is used for the labeling of proteins or peptides.
- Bromoacetamido group of BABE reacts with cysteine residues under physiological conditions and introduces a chelator into the proteins or peptides. Iron chelate of the BABE (Fe-BABE) attached on a protein can cut a peptide or a DNA chain spatially closed to the chelate moiety. The cleavage site is within 12 angstroms from a FeBABE binding site. Therefore, FeBABE is a unique tool for determining a three dimensional structure of a protein and a binding structure of a protein-DNA complex.
- The cleavage reaction of a peptide or a DNA chain by the iron (II)-chelate, which is generated by the reduction of iron (III)-chelate with ascorbic acid, is performed in the presence of hydrogen peroxide. Since the cleavage reaction completes very quickly, 10 seconds to 20 min incubation time is sufficient. Cleavage mechanism of a peptide by Fe-BABE is shown in figure 1.[1)]

So far, information of a three dimensional structure of *E. coli* cytochrome bd quinol oxidase and a structure of a subunit of *E. coli* RNA polymerase were obtained by Fe-BABE. Dr. Owens and Dr. lshihama, *et al* applied FeBABE as a chemical nuclease for the mapping the promoter DNA sites. They prepared single- cysteine mutants of σ^{7O} protein and introduced Fe-BABE into these mutants to investigate the orientation of the σ^{7O} protein and the promoter DNA. [1)]

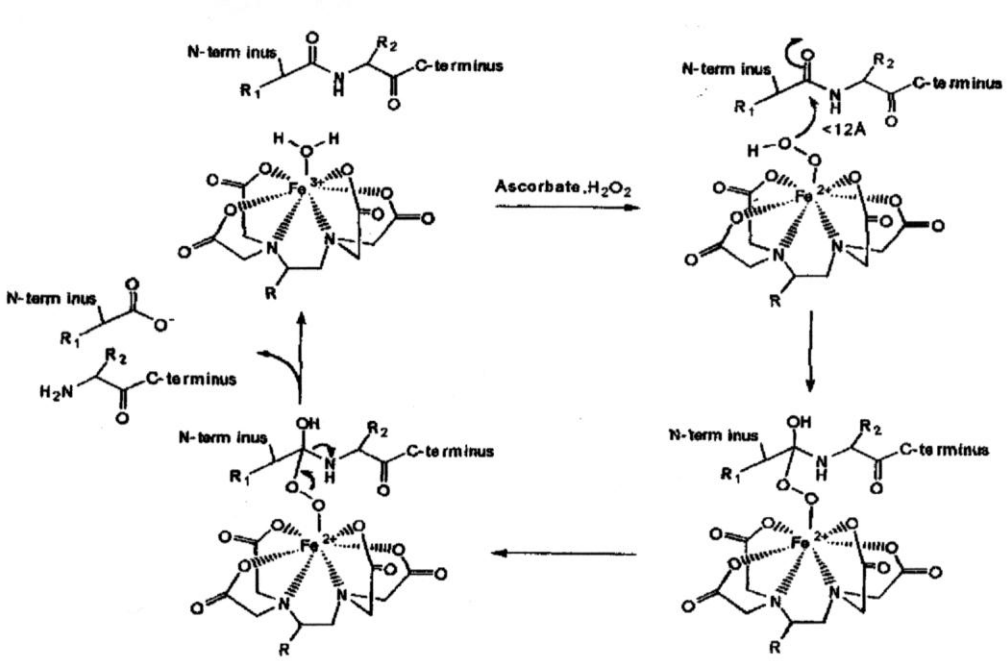

Fig.1 Reaction mecanism of FeBABE

Applications / Use

FeBABE allows new and various applications. Uptima recommends to see the litterature for information of uses.

Labeling Procedure
1. Dialyze the protein solution in conjugation buffer (10-20 mM MOPS, 0.2 M NaCl, 2 mM EDTA, 5% glycerol, pH 8.0) at 4 ºC overnight.
2. After dialysis, adjust the protein concentration to 15-30 mM.
3. Add 15 µl of 20 mM FeBABE DMSO solution to 1 ml of the protein solution, and incubate it at 37 ºC for 1 h. The final concentration of FeBABE is 0.3 mM (10-20X excess to the protein)
4. Dialyze the reaction mixture in protein storage buffer (10-20 mM Tris, 0.1-0.2 M KCl, 10 mM $MgCl_2$, 0.1 mM EDTA, 50% glycerol, pH 7.6) at 4 ºC overnight.

Propocol for FeBABE derivatization of a Cys-mutated protein (Colland 1999)

Each purified protein preparation in storage buffer [10 mM Tris pH 7.6, 10 mM MgCl2, 0.1 mM EDTA, 1 mM dithiothreitol, 0.2 M KCl and 50% (v/v) glycerol] was dialysed overnight at 4°C against the conjugation buffer [10 mM MOPS pH 8.0, 2 mM EDTA, 0.2 M NaCl and 5% (v/v) glycerol].

Conjugation was performed by mixing 300 µM of FeBABE with 20 µM of each mutant for 4 h at room temperature. This mixture was then dialysed at 4°C against conjugation buffer to remove free FeBABE.

One should include a control, a protein devoided of cysteine residue (wild-type protein) that is incubated with FeBABE (no DNA cleavage should be observed, showing FeBABE cleavage specificity and that the non-covalently bound FeBABE is totally removed by dialysis).

One could also modifiy with FeBABE tha protein that is unfolded by dialysis at 4°C into the conjugation buffer containing 6 M urea, then mixed with FeBABE (300 µM final) for 4 h at room temperature. After dialysis at 4°C against the conjugation buffer to refold the protein and to remove free FeBABE, on could get additionnal structural datas.

The concentration of free cysteine can be determined on both unconjugated and conjugated proteins with the fluorescent CPM reagent (Greiner et al., 1997), or with the DTNB reagent (UP01566).

A systematic mapping of the contact sites of various transcription factors on the RNA polymerase was allowed with a modified method of FeBABE conjugation to protein Lys residues by using 2-iminothiolane (2-IT) as a linker. Using the modified method, the contact sites on the core enzyme subnits of two elongation factors, NusA and GreA, and an RNA polymerase chaperone $f\ddot{O}$ were located.

FT-UP99476

Other information

Technical Notice NT-99476a:
Innovative method for the determination of contact sites for nucleic acid-to-protein or protein-to-protein interactions

Related products :

DTNB	UP01566K	Quantitation of cysteine sulfhydryls
Aminobenzyl-EDTA	T32100	for carboxylic acid labeling
Isothiocyanobenzyl-EDTA	T32110	for amine labeling
Maleimido-C3-NTA	T3212A	for thiol group labeling
AB-NTA	BE8210	for carboxylic acid labeling
DTPA Anhydride	T31100	for amine group labeling
CelluSep	Dialysis Membranes	removal of unreacted and by-products (desalting)

Literature

1) Colland, F. et al., Positioning of σ^S, the stationary phase σ factor, in *Escherichia coli* RNA polymerase-promoter open complexes; EMBO J. 1999 18: 4049-4059. [Abstract] [Full Text]

2) DeRiemer, L. H., Meares, C. *F., J. Labelled Compd. Radiopharm., 18,* 1517 (1981).

3) Ghaim, J.B., et al. (1995). Proximity mapping the surface of a membrane protein using an artificial protease: Demonstration that the quinone-binding domain of subunit I is near the N-terminal region of subunit II of cytochrome bd. Biochemistry 34:11311-11315.

4) Kedzierska B. et al., The C-terminal domain of the Escherichia coli RNA polymerase {alpha} subunit plays a role in the CI-dependent activation of the bacteriophage {lambda} pM promoter, Nucleic Acids Res., 35: 2311 - 2320 (2007) Article

5) Michael M. et al. ; Inaugural Article: Restructuring of an RNA polymerase holoenzyme elongation complex by lambdoid phage Q proteins ; PNAS 98: 8972-8978. [Abstract], [Full text]

6) Miyake, R., et al.., *Biochemistry, 37,*1344 (1998).

7) Murakami, K., e., *Proc. Natl. Acad. Sci. USA,* 94, 1709 (1997).

8) Owens J.T., et al., *Proc. Natl.. Acad. Sci. USA,* 95, 6021 (1998).

9) Owens, J.T., et al., (1998). Mapping the promoter DNA sites proximal to conserved regions of a σ70 in an Escherichia coli RNA polymerase-lacUV5 open promoter complex. Biochemistry 37:7670-7675.

10) Rana, T.M. and Meares, C.F. (1991). Transfer of oxygen from an artificial protease to peptide carbon during proteolysis. Proc. Natl. Acad. Sci. U.S.A. 88:10578-10582.

11) Traviglia, S.L., et al. (1999). Mapping protein-protein interactions with a library of tethered cutting reagents: the binding site of σ70 on Escherichia coli RNA polymersase. Biochemistry 38:4259-4265. [Abstract]

For any additionnal information, please inquire at Uptima- Interchim
address: 213 av.J.F.kennedy, 03103 Montlucon France
fax : +33 (0)4 4 70 03 82 60, hotline: +33 (0)4 70 03 76 06, e-mail uptima@interchim.com

rev.B10E