

## FeBABE

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

### Product Description

Catalog number : UP994760

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

#### Features:

- mild conjugation reaction with a peptide or a protein (to SH groups, and even NH<sub>2</sub> 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

prepare metal-conjugated biomaterials (pharmaceutical and diagnostic probes with In<sup>3+</sup>-chelated EDTA)  
study of protein-protein and protein-nucleic acids interactions

### 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.<sup>1)</sup>

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. Ishihama, *et al* applied FeBABE as a chemical nuclease for the mapping the promoter DNA sites. They prepared single- cysteine mutants of  $\sigma^{70}$  protein and introduced Fe-BABE into these mutants to investigate the orientation of the  $\sigma^{70}$  protein and the promoter DNA.<sup>1)</sup>

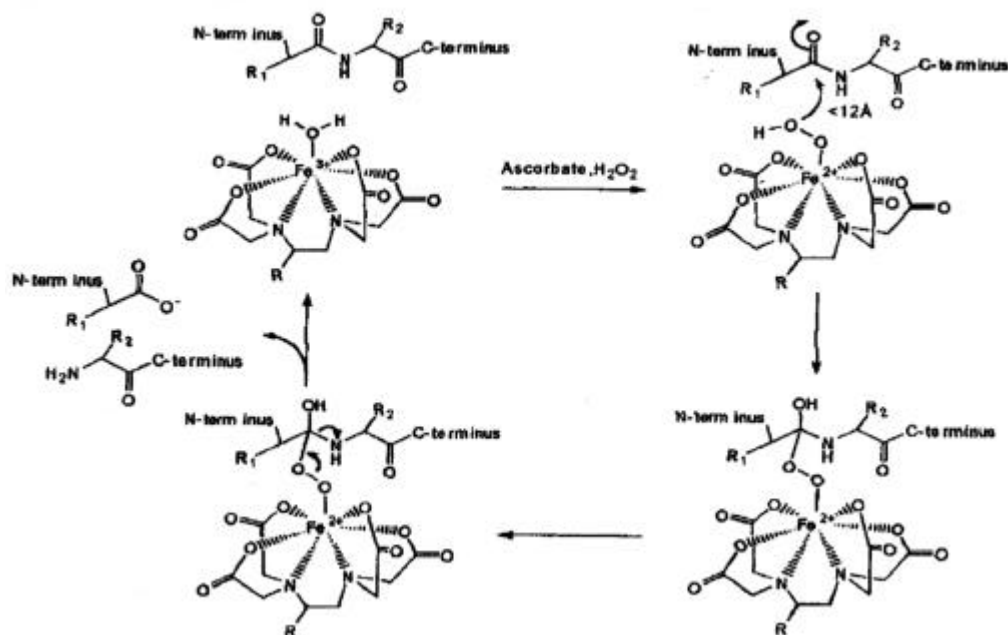


Fig.1 Reaction mechanism of FeBABE

## Directions For Use

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

### Protocole 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 MgCl<sub>2</sub>, 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 modify with FeBABE the 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, one could get additional 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 subunits of two elongation factors, NusA and GreA, and an RNA polymerase chaperone *f*0 were located.

## Other Information

Technical Notice [NT-UP99476](#):

Innovative method for the determination of contact sites for nucleic acid -to-protein or protein-to-protein interactions

### Related products :

UP01566K DTNB quantitation of cysteine sulfhydryls  
 CelluSep Dialysis Membranes removal of unreacted and by-products (desalting)

### Literature

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  - 2) 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.
  - 3) Murakami, K., Kimura, M., Owens, J. T., Meares, C. F., Ishihama, A., *Proc. Natl. Acad. Sci. USA*, 94, 1709 (1997).
  - 4) Miyake, R., Murakami, K., Owens, J. T., Greiner, D. P., Ozoline, O. N., Ishihama, Meares, C. F., *Biochemistry*, 37,1344 (1998).
  - 5) Owens J.T., Miyake R., Murakami K., Chmura A.J., Fujita N., Ishihama A., Meares C.F., *Proc. Natl. Acad. Sci. USA*, 95, 6021 (1998).
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  - 7) Owens, J.T., et al., (1998). Mapping the promoter DNA sites proximal to conserved regions of a  $\sigma 70$  in an Escherichia coli RNA polymerase-lacUV5 open promoter complex. *Biochemistry* 37:7670-7675.
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