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
Packaging : 1mg
Appearance: yellowish brown powder
Storage : –20°C
Purity: > 95.0% (HPLC)
Shipping : Room Temperature
Solubility: 1 mg/ml in H2O, >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. Ishihama, et al applied FeBABE as a chemical nuclease for the mapping the promoter DNA sites. They prepared single- cysteine mutants of σ70 protein and introduced Fe–BABE into these mutants to investigate the orientation of the σ70 protein and the promoter DNA. 1)
Applications / Use

FeBABE allows new and various applications. Uptima recommends to see the literature 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 °C 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 °C 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 MgCl₂, 0.1 mM EDTA, 50% glycerol, pH 7.6) at 4 °C 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 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 data.

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 j/ were located.

Contact your local distributor Uptima, powered by  Uptima@interchim.com
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 sulphydryls
- 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 σ70, the stationary phase σ34 factor, in Escherichia coli RNA polymerase-promoter open complexes; EMBO J. 1999 18: 4049-4059. [Abstract] [Full Text]
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]

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