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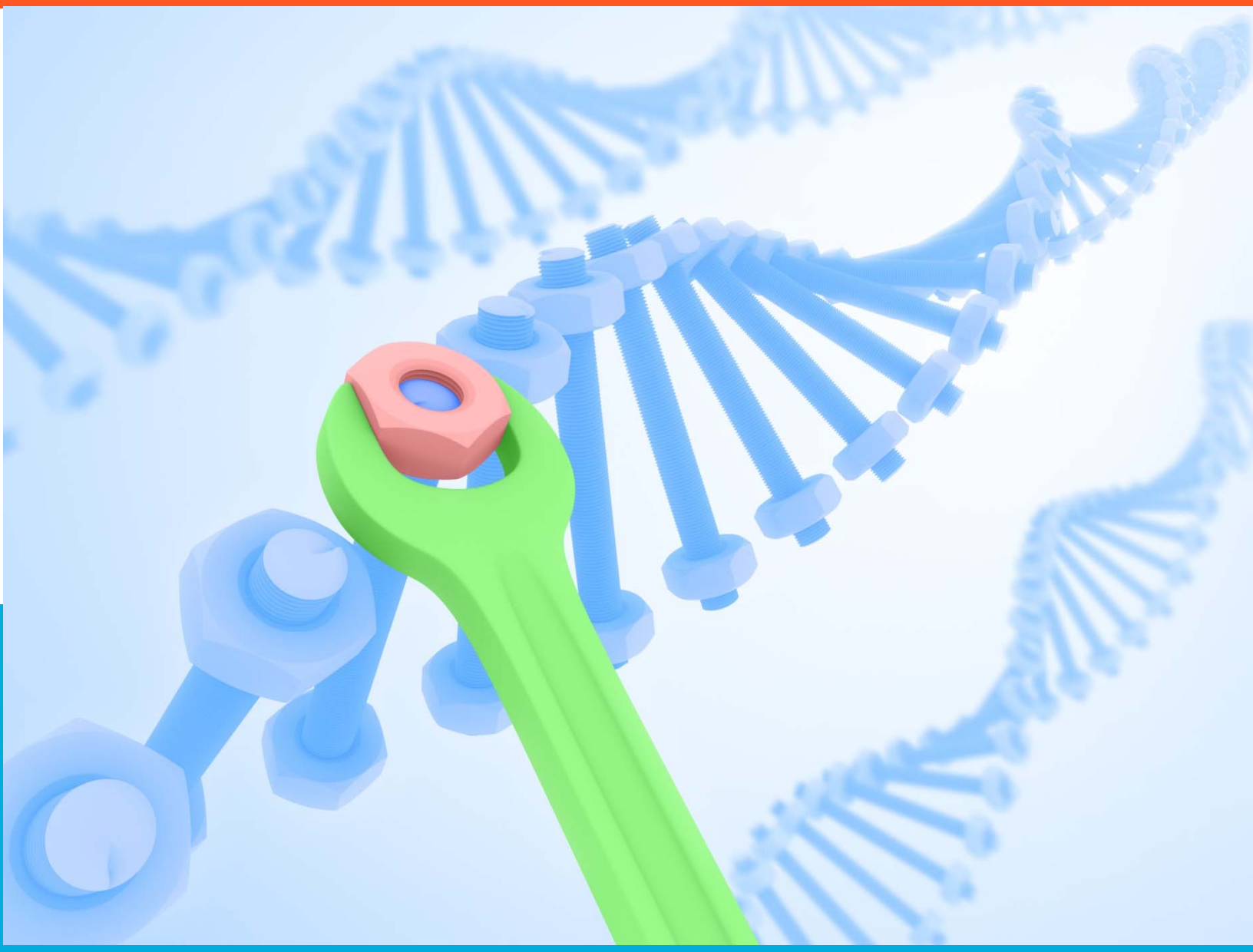

EK Standard Kit C91000-0001

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Description

Recombinant Enterokinase, 6xHis -tagged (rEK, 6xHis) is a highly purified preparation of the catalytic subunit of bovine enterokinase with a 6xHis tag at the C-terminus, which recognizes the identical cleavage site as the native enzyme, AspAspAspAspLys(DDDDK), and has similar enzymatic activity. rEK, 6xHis exhibits superior rates of cleavage of fusion proteins containing the recognition sequence when compared to the native enzyme (1). Biobasic rEK, 6xHis is purified to near homogeneity and, unlike some preparations of native bovine enterokinase, exhibits no secondary cleavage arising from contaminating proteases. The preparation is also functionally tested for activity with fusion proteins, and is an ideal choice for rapid removal of N-terminal fusions and production of recombinant proteins virtually free of vector-encoded sequences.
A Cleavage Control Protein is included in each kit for conducting control digests in parallel with experimental samples, or to test cleavage under customized buffer conditions. rEK, 6xHis cleaves the 39kDa Cleavage Control Protein into two proteolytic fragments of 22 kDa and 17 kDa, each of which are easily visualized by standard SDS-PAGE followed by Coomassie blue staining.

Components

Each kit (C91000-0001) contains the following components sufficient for 20 Assays

- 250 U Recombinant Enterokinase, 6xHis -tagged
  (in 50 mM Tris-HCl, pH 8.0, 0.5 M NaCl and 50 % glycerol)
- 20 x 50 µg Cleavage Control Protein
  (lyophilized from 25 mM Tris-HCl, pH 7.4, 50 mM NaCl)
- 1 ml 1 x rEK, 6xHis Dilution/Storage Buffer
  (50 mM Tris-HCl, pH 8.0, 0.5 M NaCl and 50 % glycerol)
- 1 ml 10 x rEK, 6xHis Cleavage Buffer
  (250 mM Tris-HCl, pH 7.4, 500 mM NaCl, 20 mM $CaCl_2$,)

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Storage

Store the kits at -80°C.

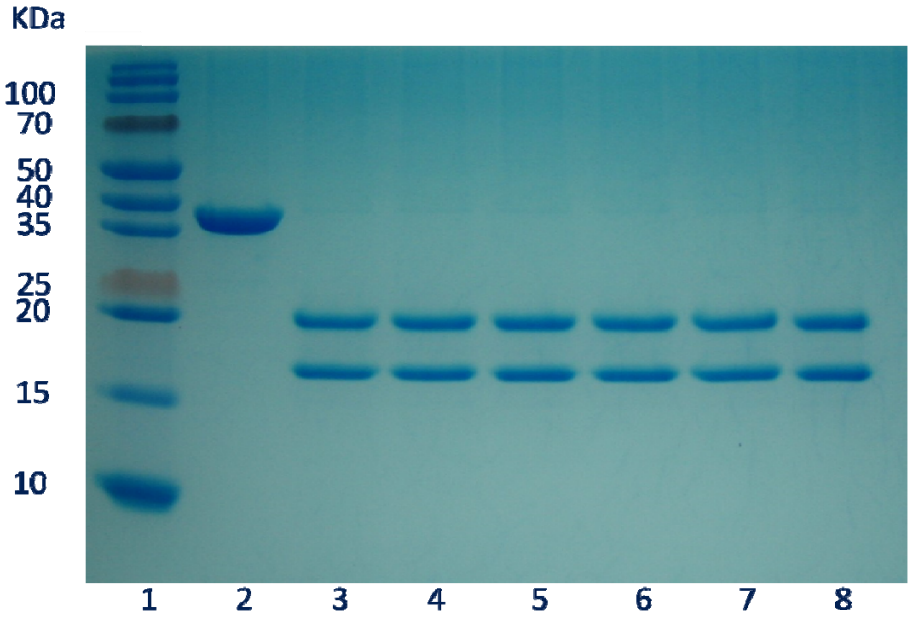
Factors that influence rEK, 6xHis activity

Depending on the buffer used and its chemical components, rEK, 6xHis cleavage efficiency may be affected. Perform pilot digests with the Cleavage Control Protein to evaluate the effect of the buffer used. Table 1 shows how rEK activity is influenced by various conditions and components.

Table 1

Condition or Component	Effect on rEK, 6xHis activity
< 0.5% Triton X-100	None
< 0.5% TWEEN 20	None
< 100 mM DTT	None
> 250 mM NaCl	Inhibitory
> 250 mM Imidazole	Inhibitory
> 1 mM PMSF	Inhibitory
> 2 M Urea	Inhibitory
> 2 M Guanadine	Inhibitory
0.0625% SDS	Secondary cleavage

rEK, 6xHis Digestion Consistency



As shown here, our rEK, 6xHis shows very consistent 95% digestion efficacy through various batches. 1unit of rEK used per lane per reaction.

Lane 1: Protein Marker
Lane2: Fusion Protein (Substrate)
Lane 3: Lot# DA27DB0005
Lane 4: Lot# F117DA0001
Lane 5: Lot# F328DA0001
Lane 6: Lot# F328DA0002
Lane 7: Lot# F612DA0001
Lane 8: Lot# F913DA0001

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rEK, 6xHis Cleavage

One unit of Recombinant Enterokinase, 6xHis -tagged (rEK, 6xHis) is defined as the amount of enzyme that cleaves95% of 50 µg Cleavage Control Protein in 50 mM NaCl, 25 mM Tris-HCl, 2mM $CaCl_2$, pH 7.4 at 25°C in 16 h. Because each target protein presents the cleavage site somewhat differently, we recommend testing several enzyme-to-target protein ratios, concentrations, temperatures, and incubation times to optimize the efficiency of cleavage. Incubation temperatures ranging from 4–37°C can be used. We recommend starting at room temperature (20–25°C), as rEK, 6xHis is most active at this temperature. Optimal specificity is achieved by using the lowest amount of rEK necessary to achieve complete cleavage. Excess rEK, 6xHis may result in unwanted proteolysis at secondary sites.

Small Scale Optimization

The following protocol is an example of a simple optimization experiment designed to estimate the appropriate ratio of rEK to target protein. In this approach, a constant amount of protease is added to three different amounts of target protein. Samples are analyzed at increasing incubation times. This example represents rEK, 6xHis:target protein ratios (unit/µg) of 1:20, 1:50, and 1:100.

Note: An rEK, 6xHis:target protein ratio (unit/µg) of 1:50 incubated at room temperature for 16 h is appropriate for most applications.

1. Dilute rEK,6xHis in 1 x rEK, 6xHis Dilution/Storage Buffer to a concentration of 1-unitrEK per 1 µl. Dilutions can be stored in this buffer at -20°C for several weeks. The concentration of rEK, 6xHis (units/µl) is provided on the tube label.

2. Assemble the following components in three separate 1.5ml tubes:

10 x rEK, 6xHis Cleavage Buffer	10µl	10µl	10µl	10µl
Cleavage Control Protein	50ug	NONE	NONE	NONE
Target Protein		20ug	50ug	100ug
Diluted rEK, 6xHis (1 unit/µl)	1 µl	1 µl	1 µl	1 µl
ddH_2O	x µl	x µl	x µl	x µl

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Total volume	100 µl	100 µl	100 µl	100 µl

3. Incubate reactions at room temperature. Remove 10 µl samples after 0, 2, 4, 8, and 16 h. Add 2 µl of 5 x SDS sample buffer to each 10 µl sample. Store at -20°C until SDS-PAGE analysis.

4. Determine the extent of cleavage of the samples by SDS-PAGE analysis. Run an undigested sample of the target protein as a control.

Important: If unexpected or secondary cleavage appears, please Incubate reactions at 2-8°C overnight, and rEK, 6xHis:target protein ratio may be increased.

Scale-Up Reaction

When a satisfactory condition for the small-scale reaction is found, increase the size of the reaction proportionately. If the reaction volume is kept in proportion to the example above, a relatively large volume will be required for digestion of 1 mg of target protein.

1. Prepare 1 x Cleavage Buffer by diluting 10 x Cleavage Buffer with sterile water. Chill at 4°C prior to use.
2. Add rEK, 6xHis to target protein at the desired rEK, 6xHis:target protein ratio and incubate at room temperature for the desired time.

3. Analyze samples by SDS-PAGE analysis.

Important: If unexpected or secondary cleavage appears, please Incubate reactions at 2-8°C overnight, and rEK, 6xHis:target protein ratio may be increased.

Inactivation of rEK, 6xHis

Inactivation of any residual rEK, 6xHis activity may be desired in some applications. Serine protease inhibitors will inactivate rEK, 6xHis. For example, APMSF (Calbiochem) is a water-soluble suicide substrate derived from PMSF that inactivates rEK, 6xHis by covalent attachment to the active site serine residue. A working stock can be prepared in water at 50 mM (500x), and is stable for 1-3 months.

Note:Protease inhibitors are very toxic. Please use caution with these products and carefully follow the manufacturer's recommendations for use.

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References

1.Collins-Racie, L. A., McColgan, J. M., Grant, K. L., DiBlasio-Smith, E. A., McCoy, J. M., and LaVallie, E. R. (1995) Bio/Technology 13, 982-987.

Terms and Conditions

✓ Orders and Deliverables

Bio Basic will deliver the agreed upon quantity of purified target protein, in the chosen expression system, to the customer. Final product will be stored in regular buffer (Tris-HCl, PBS etc). Along with the final purified protein, Bio Basic will provide related quality documentation as outlined in the protein order form. All documentation will be sent in hard copy with the final product and electronically via email.

No protein expression and purification project are initiated without a purchase order or credit card number. Bio Basic accepts credit card (Visa or MasterCard), cheque or wire as payment methods. Payment term is net 30 days. A 50%, non-refundable, start up fee may be required upon project initiation.

✓ Turnaround

Bio Basic will make its best effort to ship the purified protein as soon as it is ready but the outlined turnaround time is an estimate and the actual time may vary depending on the project. Bio Basic will provide the customer with an estimate of the completion date before initiating the project.

✓ E. coli guaranteed packages

The E. coli guaranteed packages have the following features:

i) The customer provides the plasmid or the expression strain; or the gene is synthesized by Bio Basic for an additional charge.

ii) The size of the target protein is usually <100KDa.

iii) In some rare cases, in order to facilitate expression, Bio Basic may try different tags and the final protein product may contain such tag.

iv) The final purified protein is tag-containing.

v) Bio Basic does not guarantee the endotoxin level of the purified protein.

vi) Bio Basic guarantees the protein to be soluble, but does not guarantee the bioactivity or functionality. If the target protein expresses as an inclusion body, refolding will be done to make it soluble.

vii) If Bio Basic fails to deliver the guaranteed amount due to the nature of the protein or other unpredictable circumstances, only the set-up fee will apply.

✓ Protein Purification Packages

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The protein purification packages have the following features:
i) The customer provides the plasmid or the expression strain; or the gene is synthesized by Bio Basic for an additional charge.
ii) The size of the target protein is usually <100KDa.
iii) In some rare cases, in order to facilitate expression, Bio Basic. may try different tags and the final protein product may contain such tag.
iv) The final purified protein is tag-containing.
v) Bio Basic does not guarantee the endotoxin level of the purified protein.
vi) Bio Basic guarantees the protein to be soluble, but does not guarantee the bioactivity or functionality. If the target protein expresses as an inclusion body, refolding will be done to make it soluble.
vii) If Bio Basic fails to deliver the guaranteed amount due to the nature of the protein or other unpredictable circumstances, only the set-up fee will apply.

✓ Protein Analysis

Standard QC methods include SDS-PAGE and Western Blot (if desired). Western-blot can only analyze seven samples each time in addition to controls and markers. Bio Basic will provide one free Western-blot analysis. If customer requires more than one, extra charges will apply. Bio Basic will provide the primary anti-His-tag antibody. If an antibody other than the anti-His-tag antibody is required, the customer needs to provide Bio Basic with the specific antibody. There are many factors affecting Western-blot analysis, Bio Basic cannot guarantee that there is not a false positive/negative result. If the result is abnormal, Bio Basic can provide another Western-blot analysis free of charge.

✓ Proteins that fail to be expressed and purified

Bio Basic makes its best effort to express and purify the target protein. Should Bio Basic be unsuccessful in purifying the target protein (due to sequence instability, toxicity, hydrophobicity, etc.), the minimum 50%, non-refundable, start-up fee will apply.

✓ Cancellations

For any protein, the customer may cancel the order after reaching an agreement with Bio Basic:
i) If Bio Basic has received the order but has not initiated the project; the customer is entitled to cancel of the order without any penalty.
ii) If Bio Basic has prompted protein expression and purification; the customer may cancel the order, however, the customer is obligated to pay a penalty cost depending on the stage or status of the project.
iii) If Bio Basic has obtained the target protein and completed the project; the customer may cancel the order, however, the full amount will be paid to Bio Basic.

✓ Patents

Bio Basic serves as a service provider and offers synthesis service of sequences provided by the customer. It is the sole responsibility of customer to verify whether his respective work is the result of any infringements of any patents. Bio Basic. expressly disclaims any liability in this regard.

✓ Inspection Policy

Upon receipt of shipped goods, customer shall inspect the shipment promptly for damages, shortages and correct identity of the product. Any product that is not identical to the requested

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protein will be replaced or authorized for return and credit, at our option. Any claims must be submitted within 10 days of shipment.

- ✓ **Warranty**

Bio Basic guarantees 100% accuracy and match of the protein requested. Any claims must be submitted within 3 months of shipment. Bio Basic reserves the right to refuse handling disputes after a period of 3 months.

- ✓ **Confidentiality**

Bio Basic and the customer agree that all confidential information including but not limited to project scope, contract terms, pricing information, product development technologies and processes, and business-related information shall not be disclosed to any third party and both parties receiving confidential shall only make internal use of the confidential information.

- ✓ **Usage**

Bio Basic products are used exclusively for scientific research purposes only. Materials will not be used for human or animal consumption.

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More Services/Products

Thank you for your interest in Bio Basic Canada!

As one of the world leaders in serving science, we are confident that our product has the highest quality with the most affordable price in the market.

We promise to beat the price of any quotes issued by our competitors. If you send us a **formal quotation** from our competitor that is offering cheaper service than us, **we will beat it**.

Besides DNA Sequencing, we also provide a wide variety of other services, including **Gene Synthesis, Oligo Synthesis, Peptide Synthesis, Protein Expression and Purification, and Antibody Synthesis**, as well as **products**, including**Tissue Culture ware, Molecular Biology Kits, Lab Equipment, PCR-Related Products, and more**. Please inquire if you are interested in any of the above **services/products** to learn the promotions that we have at the moment.

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