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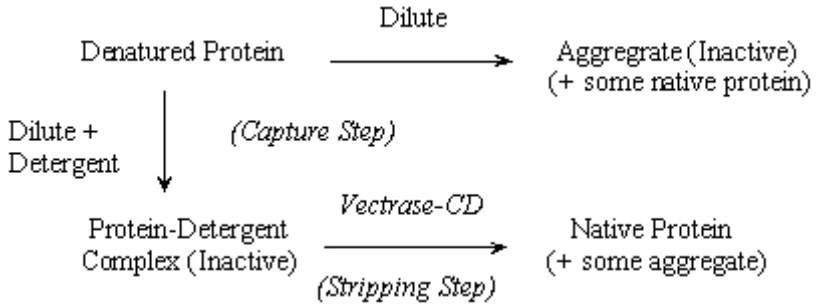
Vectrase™-CD

Cat. No.: 3000 **Storage: Store at 2-8°C.**

Protein production by recombinant methods in *E. coli* or other bacterial systems often results in unfolded or misfolded proteins as inclusion bodies. There is an advantage to having inclusion bodies since they are easy to purify. Once the inclusion bodies have been isolated, they must be solubilized and then refolded into the native, active state. The *Vectrase-CD* kit helps to retrieve the properly folded protein.

The theory of the *Vectrase-CD* kit is more clearly illustrated in the diagram below. The top path shows how folding by dilution at high protein concentrations usually proceeds. The lower path shows the method as a two-step process. In the first step, "the Capture Step", a detergent forms a complex with protein preventing aggregation. In the second step, "the Stripping Step", the *Vectrase-CD* strips the detergent from the protein allowing it to refold. The result is not only successful folding at higher protein concentrations as compared to conventional folding by dilution but also more properly folded protein.



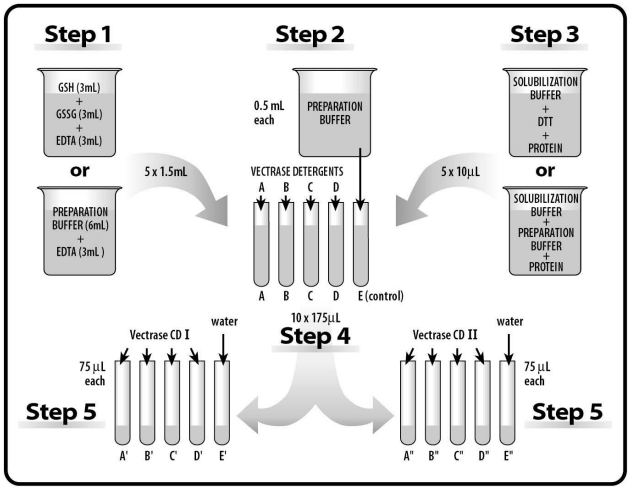
When using the *Vectrase-CD* kit for the first time, some solutions have to be prepared. Prepare solutions a and b and for proteins with disulfide bonds prepare c, d and e.

a) *Vectrase-CD* I solution. Add 15 mL of deionized water. Make 1.5 mL aliquots, flush with an inert gas (N_2 or Ar.), and store at -15 ± 5°C.
b) *Vectrase-CD* II solution. Add 15 mL of deionized water. Make 1.5 mL aliquots, flush with an inert gas (N_2 or Ar.), and store at -15 ± 5°C.
c) DTT solution. Add 1 mL of preparation buffer to the solid DTT. Make 100 µL aliquots, flush with an inert gas (N_2 or Ar.), and store at -15 ± 5°C.
d) GSH solution. Add 50 mL of preparation buffer to the solid GSH. Make 5 mL aliquots, flush with an inert gas (N_2 or Ar.), and store at -15 ± 5°C.
e) GSSG solution. Add 50 mL of preparation buffer to the solid GSSG. Make 5 mL aliquots, flush with an inert gas (N_2 or Ar.), and store at -15 ± 5°C.

The following protocol is written for a denatured protein and involves screening of four different *Vectrase* detergents and two *Vectrase-CDs*. Some optimizing is needed for best results. Once the optimum condition is determined, the methodology can be applied to routine protein folding.

The protocol is written for proteins with disulfide bond(s). For proteins without disulfide bond(s), see underlined text.

Protocol



1. a) Prepare dilution buffer (See Notes 1 & 2):
   Mix 3 mL of the following GSH reconstituted solution
   GSSG reconstituted solution
   EDTA solution

   <u>For proteins without disulfide bonds (See Note 1), mix 6 mL of preparation buffer with 3 mL of EDTA solution.</u>

   b) Transfer 1.5 mL aliquots of the dilution buffer to five test tubes, and label the tubes A to E.

2. Add 0.5 mL of detergent solution to the corresponding tubes (*i.e. Vectrase* Detergent A to tube A, *Vectrase* Detergent B to tube B, *Vectrase* Detergent C to tube C and *Vectrase* Detergent D to tube D). Add 0.5 mL of the preparation buffer to tube E as a control. Properly mix the solutions.

3. a) Solubilize the inclusion bodies with the solubilization buffer provided. (1 - 50 mg of inclusion bodies per mL of buffer has been suggested). Add DTT solution at 1/9 the volume of solubilization buffer used. (<u>For proteins without disulfide bonds, add preparation buffer instead of DTT solution</u>) Denature following the usual time and temperature conditions.

   (Optional). Determine the protein concentration for your reference.

4. Label two new sets of five test tubes: A' to E' and A" to E". Transfer 175 µL of the diluted protein solution to the corresponding tubes (*i.e.* protein solution A to tubes A' and A", protein solution B to tubes B' and B" *etc.*). Properly mix the solutions.

5. Add 75 µL of *Vectrase-CD* I to A' to D', *Vectrase-CD* II to A" to D", and deionized water to E' and E". (See Note 3)

Allow the samples to refold overnight and measure the protein folding yield.

<u>Notes</u>

1. The amount of dilution solution used depends on the dilution factor. A dilution factor from 10 to 1000 has been used. For initial screening, a dilution factor of 200 is recommended (*i.e.* 10 µL of denatured protein sample is to be diluted with 2 mL of solution).

2. The suggested protocol uses a GSH:GSSG mole ratio of 1, this ratio might have to be optimized for best results.

3. For best results, the optimum volume ratio of *Vectrase-CD* to diluted protein solution might have to be adjusted. Initially, a volume ratio of 3:7 is suggested.

<u>References</u>

1. Daugherty, D., Rozema, D., Hanson, P., and Gellman S. *J. Biol. Chem.* 1998, **273,** 33961-33971.
2. Kurganov B.I., and Topchieva I.N., *Biochemistry (Moscow)* 1998, **63,** 413-419.
3. US Patent 5563057**.**

INS3000-0
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b) Add 10 µL of the solubilized protein solution from Step 3(a) to each tube prepared in Step 2. This is the diluted protein solution. Properly stir the samples for at least 10 minutes.