

S•Tag[™] rEK Purification Kit

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About the Products and Vectors

Description

The S•Tag™ rEK Purification Kit is designed for rapid affinity purification of S•Tag fusion proteins produced using many Novagen® vectors. S-protein Agarose specifically retains S•Tag fusion proteins (1–3). When fusion proteins are expressed from vectors that also encode an enterokinase cleavage site (AspAspAspAspLys↓) between the S•Tag sequence and the cloning region, target protein can be released from S-protein Agarose by digestion with Recombinant Enterokinase (rEK). The standard protocol involves batch-wise binding to S-protein Agarose, washing, rEK treatment, and capture of rEK with EKapture™ Agarose, leaving purified target protein in solution. Under these gentle conditions, homogeneous target proteins lacking the S•Tag peptide are recovered and the S-protein Agarose cannot be reused.

As an alternative, fusion protein can be eluted from S-protein Agarose under harsh conditions that disrupt the S•Tag:S-protein interaction (e.g., 3 M guanidine thiocyanate, 3 M magnesium chloride, or 0.2 M citrate pH 2). In this case, S•Tag peptide remains attached to the protein and the S-protein Agarose can be recycled. If the target protein accumulates in bacterial cells as inclusion bodies, insoluble fraction can be prepared and dissolved in 6 M urea. Target proteins can bind S-protein Agarose in the presence of 2 M urea and elute either with rEK digestion in presence of urea, or with the partially denaturing conditions mentioned above.

Binding can be performed in either column or batch mode. The capacity of S-protein Agarose depends in part on size and folding characteristics of a given target protein. When tested under gentle rEK-mediated elution conditions using S•Tag β-galactosidase, minimum binding capacity of the resin is 500 µg/ml. The amount of cell extract required for purification also depends on expression level of the target protein.

Components

The S•Tag™ rEK Purification Kit contains sufficient resin, enterokinase, and EKapture™ Agarose to purify up to 1 mg target protein under standard conditions.

- 2 ml S-protein Agarose (4 ml of a 50% slurry in 150 mM NaCl, 50 mM Tris-HCl, 1 mM EDTA, 0.02% sodium azide, pH 7.5)
- 3 x 5 ml 10X Bind/Wash Buffer (1.5 M NaCl, 200 mM Tris-HCl, 1% Triton® X-100, pH 7.5)
- 2 ml 1X rEK Dilution/Storage Buffer (200 mM NaCl, 20 mM Tris-HCl, 2 mM CaCl₂, 50% glycerol, pH 7.4)
- 50 U Recombinant Enterokinase (in 200 mM NaCl, 20 mM Tris-HCl, 2 mM CaCl₂, 50% glycerol, pH 7.4)
- 1.5 ml EKapture Agarose (3 ml 50% slurry in phosphate buffer, 500 mM NaCl, 0.02% Sodium azide, pH 7.3)
- 2 Spin Filters, 5 ml capacity

Storage

Store Recombinant Enterokinase (rEK) and 1X rEK Dilution/Storage Buffer at –20°C. Store remaining components at 4°C. **Do not freeze S-protein Agarose or EKapture™ Agarose.**

Caution: S-protein Agarose and EKapture Agarose are supplied in a buffer containing 0.02% Sodium azide. Handle with caution. Wear gloves and appropriate laboratory clothing.

Related products/available separately	Size	Cat. No.
S-protein Agarose	2 ml	69704-3
	5 × 2 ml	69704-4
Recombinant Enterokinase	50 U	69066-3
EKapture™ Agarose	1.5 ml	69068-3
Spin Filters, 5-ml capacity	pkg/2	69074-3
Perfect Protein™ Markers, 15–150 kDa	100 lanes	69149-3
Perfect Protein Markers. 10–225 kDa	100 lanes	69079-3
Perfect Protein Western Markers	25 lanes	69959-3
S•Tag™ Thrombin Purification Kit		69232-3
S•Tag Rapid Assay Kit	100 assays	69212-3
FRETWORKS™ S•Tag Assay Kit	100 assays	70724-3
S•Tag Western Blot Kits		
AP (colorimetric)	25 blots	69213-3
AP LumiBlot™ (chemiluminescent)	25 blots	69099-3
HRP LumiBlot (chemiluminescent)	25 blots	69058-3
S-protein AP Conjugate	50 µl	69598-3
S-protein HRP Conjugate	50 µl	69047-3
S•Tag™ Monoclonal Antibody	50 µg	71549-3
Thrombin, Restriction Grade	50 U	69671-3
Biotinylated Thrombin	50 U	69672-3
D-Tube™ Dialyzers	Various	See User Protocols TB422 and TB495

Preparation of Cell Extracts

Target protein may be expressed in *E. coli*, insect, or mammalian cells. S•Tag™ fusion proteins produced using reticulocyte lysate extracts, such as Single Tube Protein® System 3 (STP3®) transcription/translation reactions, can be used directly (begin protocol at Binding steps on page 5). Target protein must be expressed using a vector encoding S•Tag peptide.

For a detailed description of bacterial S•Tag expression vectors and protein expression considerations, please refer to the pET System Manual (see User Protocol TB055). For in vitro transcription/translation of S•Tag fusion proteins, please refer the STP3® kits User Protocol (TB206). For insect cell expression vectors, see User Protocols for our pIEx™ vectors (TB356), pBiEx™ vectors (TB357), BacVector® (TB216) and BacMagic™ systems (TB459). For details on mammalian cell expression vectors, see the pTriEx™ System Manual (TB250).

Note: Please visit www.merck4biosciences.com for all Technical Bulletins referenced herein.

Bacterial cell extracts (pET System)

A detailed discussion of target protein induction and expression using the Novagen® pET System can be found in the pET System Manual User Protocol (see User Protocol TB055). Expression of S•Tag fusion proteins can be detected using S-protein AP or HRP Conjugate, or S•Tag Monoclonal Antibody. Additionally, S•Tag fusion proteins can be quantified rapidly in crude cell extracts using the S•Tag Rapid Assay Kit or FRETWorks™ S•Tag Assay Kit (see User Protocols TB082 and TB251, respectively).

Soluble and Insoluble Fractions

Many target proteins are expressed in both soluble and insoluble forms. BugBuster® Protein Extraction Reagent or PopCulture® Protein Extraction Reagent can be used to extract soluble proteins (see User Protocols TB245 or TB323, respectively). For viscosity reduction and enhanced recovery, Benzonase® Nuclease, rLysozyme™ Solution, or Lysonase™ Bioprocessing Agent can be added to samples (see User Protocols TB261, TB334, or TB361, respectively). Soluble protein fractions can also be prepared using standard mechanical lysis protocols or enzymatic methods, with addition of rLysozyme™ Solution, Benzonase Nuclease, or Lysonase Bioprocessing Agent.

Insoluble (inclusion body) protein fractions can be prepared using BugBuster Reagent or standard mechanical lysis protocols in combination with enzymatic methods, including rLysozyme Solution, Benzonase Nuclease, or Lysonase Bioprocessing Agent.

Insect cell extracts

Extracts can be prepared using CytoBuster™ Protein Extraction Reagent (see User Protocol TB306), Insect PopCulture® Extraction reagent (see User Protocol TB344), or standard mechanical lysis protocols.

Mammalian cell extracts

Extracts can be prepared using CytoBuster Protein Extraction Reagent (see User Protocol TB306), or standard mechanical lysis protocols.

Note: If cell extracts are stored prior to purification, centrifuge at 14,000 × g for 5 min at 4°C to remove any aggregates that may have formed during storage.

Note: Please visit www.merck4biosciences.com for all Technical Bulletins referenced herein. All User Protocols referenced herein are available at www.novagen.com.

Purification

Binding

1. Gently suspend S-protein Agarose by inversion and add 2 ml slurry (equivalent to 1 ml settled resin) to soluble protein extract. (If purifying target protein from solubilized inclusion bodies, pre-equilibrate resin with an equal volume of 1X Bind/Wash Buffer containing 4M urea prior to mixing protein extract and resin.) Transfer resin using a 1 ml capacity wide-mouth pipet tip. Mix thoroughly and incubate at room temperature on an orbital shaker at slow setting for 30 min. Do not shake vigorously, as this may denature protein.
2. Centrifuge the entire volume at 500 x g for 10 min. Carefully decant supernatant.
3. Resuspend S-protein Agarose (containing bound S•Tag™ fusion protein) in 5 ml 1X Bind/Wash Buffer. (Include 2 M urea if using partially denatured protein.) Mix by gentle vortexing or repeated inversion. Avoid vigorous vortexing.
4. Repeat steps 2 and 3 twice more to wash away unbound proteins. Remove final supernatant and elute target protein using either rEK or using GuSCN, pH, or MgCl₂ as described on page 4.

Elution with rEK

1. Resuspend S-protein Agarose/target protein in a final volume of 2 ml 1X Bind/Wash Buffer (Add 2 M urea if purifying proteins from solubilized inclusion bodies.) Add 10 units rEK and incubate for up to 16 h at room temperature on an orbital shaker. Target protein released from the agarose no longer contains the S•Tag peptide. The rEK is removed with EKapture™ Agarose.

Note: rEK is fully active in Bind/Wash Buffer and in the presence of 2 M urea. The enzyme can be diluted in 1X Dilution/Storage Buffer for use in small-scale reactions. This buffer stabilizes the diluted enzyme, which can then be stored at -20°C for up to 2 weeks.

2. Thoroughly resuspend EKapture Agarose by inversion. Add 500 µl slurry to the rEK reaction. Mix thoroughly and incubate for 10 min at room temperature on an orbital shaker.

Note: EKapture™ Agarose can be added directly to resin/target protein/rEK mixture. Alternatively, it can be pre-equilibrated in 1X Bind/Wash Buffer, if desired. If purifying protein from insoluble fraction, urea should be added to EKapture Agarose to a final concentration of 2 M prior to mixing with sample. If not using a Spin Filter to remove agarose, proceed to Step 5.
3. Transfer entire reaction to a Spin Filter that has been placed in a collection tube. Centrifuge at 500 × g for 5 min.
4. Without removing the filtrate in the lower chamber, add 1.25 ml 1X Bind/Wash Buffer to the “cake” of resin in the upper chamber, and centrifuge at 500 × g for 5 min. Filtrate contains target protein and can be used directly in many applications. See *Processing the Sample After Elution* below.
5. **Optional (if not using a Spin Filter):** Centrifuge at 500 × g for 5 min. Transfer the supernatant containing target protein to a fresh tube. Wash agarose pellet with 1–2 ml 1X Bind/Wash Buffer and centrifuge. Supernatants may be pooled.

Elution with GuSCN, pH, or MgCl₂

1. Resuspend washed resin/target protein in 1.5X settled resin volumes of one of the following elution buffers:
0.2 M citrate, pH 2*
or 3 M magnesium chloride
or 1X Bind/Wash Buffer containing 3 M guanidine thiocyanate

Incubate for 10 min at room temperature. Mix gently every few minutes to keep the resin suspended.

**Note: To make this buffer, prepare a 2 M stock of citric acid. If necessary, adjust the pH to 2.0 with 10 M KOH. Dilute to final concentration of 0.2 M citric acid.*

2. Transfer entire reaction to a Spin Filter that has been placed in a collection tube. Centrifuge at 500 × g for 5 min.
3. Without removing filtrate, add 1.25 ml elution buffer to the “cake” of resin in the upper chamber. Centrifuge at 500 × g for 5 min. Filtrate contains purified target protein.

4. **Optional (if not using a Spin Filter):** Centrifuge at $500 \times g$ for 5 min and transfer supernatant containing target protein to a fresh tube. Wash pellet with an additional 1–2 ml elution buffer and centrifuge. Supernatants may be pooled. See *Processing the sample after elution* below.
5. S-protein Agarose may be recycled by washing 3 more times with elution buffer, then 3 times with 1X Bind/Wash Buffer. Store at 4°C in 1X Bind/Wash Buffer containing 0.02 % sodium azide or other preservative.

Processing the sample after elution

Purified protein samples may be concentrated or the buffer exchanged as necessary. D-Tube™ Dialyzers are convenient for both dialysis and concentration in a single-tube format, and are available in a variety of volume capacities and molecular weight cutoffs (MWCOs). See User Protocols TB422 and TB495 for more information. Appropriate buffer and storage conditions should be determined empirically, as many proteins are prone to degradation and/or aggregation upon long-term storage (4).

References

1. Kim, J.-S., and Raines, R.T. (1993) *Protein Science* **2**, 348-356.
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