IBA-lyse
Protocol for the preparation of cleared lysates

Cat. No.: 2-1017-050, 2-1017-250

Last date of revision
June 2005

Version: PR17-0001

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Preparation of a cleared lysate after cytoplasmic expression of *Strep*-tag fusion proteins with IBA-lyse

IBA-lyse reagents are formulated for gentle disruption of *E. coli* and release of proteins. It consists of a reagent mix compatible with subsequent *Strep*-tag purification and provides a simple, rapid, inexpensive and most importantly more reproducible and milder alternative to mechanical methods such as sonication or French Press for preparation of cleared lysates to be submitted to affinity chromatography.

Material and important notes

The Kit provides:

- Buffer B which contains 1 mM EDTA, Tween 20 and avidin; store at 2-8 °C.
- Lyophilized lysozyme; store at -20 °C.
- Lysozyme reconstitution buffer; store at 2-8 °C.
- Lyophilized DNase I; store at -20 °C.
- DNase reconstitution buffer; store at 2-8 °C.

Prior first use:

- **Dissolve provided lysozyme and DNase with the respective reconstitution buffer, store reconstituted stock solutions at -20°C.**
- **Determine how much cells are intended to be lysed in the next 3 months. Per 100 ml bacterial culture, 2 ml Buffer B containing lysozyme will be needed. Add reconstituted lysozyme stock solution to the corresponding amount of Buffer B. Use 2 µl lysozyme per ml Buffer B. Store Buffer B containing lysozyme at 2-8 °C at which it will be stable for 3 months. <u>DO NOT ADD DNAse</u> at this stage! DNAse is added during the preparation after lysis of the bacterial cells.**

Notes, recommendations:

- It is recommended to work without EDTA when metalloproteins have been expressed. In this case, 1.1 mM $MgCl_2$ has to be added to Buffer B containing lysozyme prior cell lysis.
- *Strep*-tag:*Strep*-Tactin-binding is compatible with many reagents and detergents (see Table).
- 5x SDS-PAGE sample buffer: 0.25 M Tris·Cl, pH 8; 25% glycerol; 7.5% SDS, 0.25 mg/ml bromophenolblue; 12.5% v/v mercaptoethanol.

Protocol

1. **Chill Buffer B containing lysozyme at 4°C.**

2. **After bacterial cell harvest, resuspend the bacterial cell sediment with Buffer B containing lysozyme. Use 2 ml per cell sediment derived from 100 ml culture.**
   Bacterial cell sediments may be frozen at -20°C after harvest. If they are to be lysed immediately after expression/harvest, it is recommended to freeze pellets once in liquid nitrogen because freeze/thaw contributes to lysis of the bacterial wall.

3. **Incubate on ice for 30 minutes.**
   Lysis should be complete and suspension should be very viscous.

4. **Add 4 µl DNase stock solution per 2 ml Buffer B containing lysozyme and incubate on ice for 15 min.**
   Viscosity should have disappeared. Incubation can be extended up to 1 hour.

5. **(Optional) If the lysate is still viscous, add RNase A (10 µg/ml) and incubate for 15 minutes on ice.**

6. **Take a 10 µl sample for analysis of the total protein content via SDS-PAGE and/or Western blotting.**
   The 10 µl sample should be thoroughly mixed with 90 µl Buffer B and 25 µl of 5x SDS-PAGE sample buffer. Store at -20°C. The whole sample should be heated to 70°C for 10 minutes prior to SDS-PAGE. Keep in mind that the complete lysis buffer contains lysozyme (14 kDa), avidin (17 kDa per subunit), DNase I (31 kDa) and optionally RNase A (13.7 kDa).

7. **Centrifuge the suspension at 13000 rpm (microfuge) for 15 minutes at 4°C.**
   Insoluble cell components are sedimented. If the recombinant protein forms inclusion bodies it will be present in the sediment. For analysis of the insoluble part of the expressed protein, dissolve the sediment from 10 µl suspension with 125 µl 1x SDS-PAGE sample buffer (= 25 µl 5x SDS-PAGE sample buffer mixed with 100 µl Buffer B). Keep in mind that the complete lysis buffer contains lysozyme (14 kDa), avidin (17 kDa per subunit), DNase I (31 kDa) and optionally RNase A (13.7 kDa).

8. **Carefully transfer the clear supernatant to a clean tube.**
   Store the supernatant on ice until chromatography or store at -20°C if chromatography cannot be performed the same day.

9. **Take a 10 µl sample of the supernatant for analysis of the soluble part of the expressed protein content via SDS-PAGE and/or Western blotting.**
   The 10 µl sample should be thoroughly mixed with 90 µl Buffer B and 25 µl of 5x SDS-PAGE sample buffer. Store at -20°C. The whole sample should be heated to 70°C for 10 minutes prior to SDS-PAGE. Keep in mind that the lysis buffer contains lysozyme (14 kDa), avidin (17 kDa per subunit), DNase I (31 kDa) and optionally RNase A (13.7 kDa).

10. **Proceed to protocols for *Strep*-tag protein purification under native conditions in chapter "Purification of *Strep*-tag fusion proteins" of the Strep-tag manual PR02 "Expression and purification of proteins using Strep-tag and/or 6xHistidine-tag".**

Reagents compatible with *Strep*-tag:*Strep*-Tactin interaction	Concentration
Reducing Agents	
DTT	50 mM
β-mercaptoethanol	50 mM
Non-Ionic Detergents	
C_8E_4 Octyltetraoxyethylene	0.88 %
$C_{10}E_5$; Decylpentaoxyethylene	0.12 %
$C_{10}E_6$	0.03 %
$C_{12}E_8$	0.005 %
$C_{12}E_9$; Dodecyl nonaoxyethylene (Thesit)	0.023 %
DM; Decyl-ß-D-maltoside	0.35 %
LM; N-dodecyl-β-D-maltoside	0.007 %
NG; N-nonyl-β-D-glucopyranoside	0.2 %
OG; N-octyl-β-D-glucopyranoside	2.34 %
TX; Triton X-100	2 %
Tween 20	2 %
Ionic Detergents	
N-lauryl-sarcosine	2 %
8-HESO;N-octyl-2-hydroxy-ethylsulfoxide	1,32 %
SDS; Sodium-N-dodecyl sulfate	0.1 %
Zwitter-Ionic Detergents	
CHAPS	0.1 %
DDAO; N-decyl-N,N-dimethylamine-N-oxide	0.034 %
LDAO; N-dodecyl-N,N-dimethylamine-N-oxide	0.13 %
Others	
Ammonium sulfate $(NH_4)_2SO_4$	2 M
$CaCl_2$	1 M
EDTA	50 mM
Ethanol	10 %
Guanidine	1 M
Glycerol	25 %
Imidazole	250 mM
$MgCl_2$	1 M
NaCl	5 M
Urea	1 M

Note: These reagents have been successfully tested for the purification of e.g. GAPDH-*Strep*-tag with concentrations up to those mentioned. For most reagents higher concentrations may be possible, though. However, since binding depends on the sterical accessibility of *Strep*-tag in the context of the particular protein the maximal concentration may also be lower for other proteins.

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