ab173231
BSA Removal Kit Protocol

A product of Expedeon, an Abcam company

Applicable to Expedeon product codes 820-0100.

View ab173231 BSA Removal Kit datasheet:

www.abcam.com/ab173231

(use www.abcam.cn/ab173231 for China, or www.abcam.co.jp/ab173231 for Japan)

For the removal of BSA from antibodies prior to conjugation.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Bovine Serum Albumin (BSA) is often added to purified antibodies as it is an effective stabilizer. However, when labelling antibodies, the BSA becomes a hindrance, as it directly competes with the antibody to attach to the label, greatly reducing the conjugation efficiency. Therefore, prior to undertaking labelling techniques, it is essential to remove the BSA.

Common commercial BSA removal techniques can involve many laborious steps. Abcam's BSA Removal Kit (ab173231) is a simple one-step, 10-minute method which effectively separates the BSA from the antibody. The antibody is left in a suitable position for transfer to a buffer more suited to conjugation. The BSA Removal Kit can be used on any antibody sub-type, and species.

Antibodies purified using the BSA Removal Kit are fully compatible with our Lightning-Link® Antibody Conjugation kits.

2. Materials Supplied and Storage

Store at +4°C upon receipt.

Item	Quantity	Storage temperature
BSA Removal Buffer	1 vial	+4°C
Re-suspension Buffer	1 vial	+4°C

Reagents are ready to use as supplied.

3. Technical Considerations

3.1 Minimum amount of antibody to purify:

50 µg of antibody is the lower limit for seeing a clearly visible pellet.

3.2 Concentration of antibody/BSA to purify:

The BSA Removal Kit can separate BSA from antibody solutions with antibody concentrations from 0.03 mg/mL to 10 mg/mL. Separation is more efficient at higher antibody concentrations. BSA can be effectively separated when present at concentrations of up to 0.5%. If BSA is present at higher concentrations, dilute the antibody mix with de-ionized, distilled water until BSA concentration is 0.5% or less.

3.3 Buffer composition:

Buffers such as MES, Tris and PBS are compatible with the kit, and common non-buffering salts (e.g. NaCl) have no adverse effect on the separation. Glycerol up to 20% has no effect. If the glycerol content is higher than this the solution should be diluted using deionized water until the glycerol content is 20% or less. This protocol can then be followed as written.

The BSA Removal kit is effective with buffers between pH 6.0 and pH 8.0. If the buffer is outside the suggested pH range, please contact us by email (technical@abcam.com) or phone (select "contact us" on www.abcam.com for the phone number for your region).

3.4 Antibody pre-conjugation considerations:

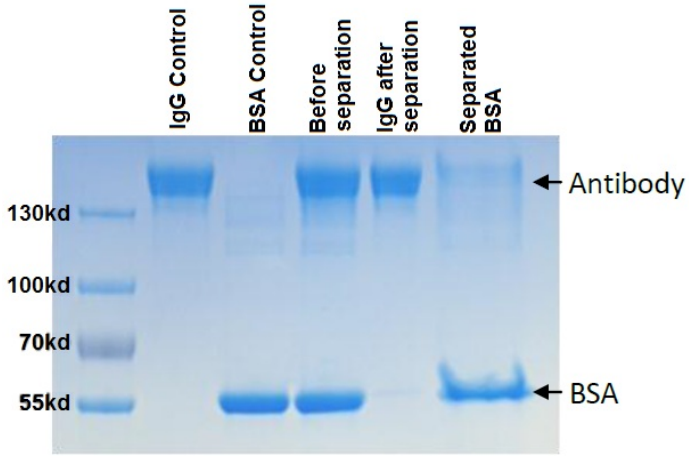
This kit can be used for preparing antibodies for conjugation. The antibody concentration for each Conjugation Kit has been optimised. Before starting the elution step of this purification procedure, please refer to the relevant Lightning-Link® Conjugation Kit datasheet or protocol for the recommended antibody concentration and find more general information about antibody conjugation at www.abcam.com/conjugationFAQs.

3.5 Contaminating products:

The BSA Removal kit can also be used to remove the following contaminants from your antibody buffer.

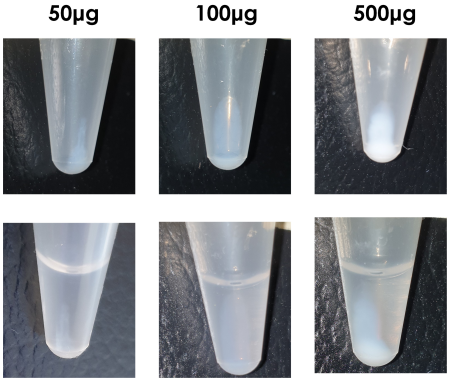
Buffer components	**Removal**
Non-buffering salts *(e.g. sodium chloride)*	✓
Chelating agents *(e.g.EDTA)*	✓
Sugars	✓
Glycerol	<20%
Thiomersal / Thimerosal	✓
Merthiolate	✓
Sodium Azide	✓
BSA	✓
Gelatin	X
Tris	✓
Glycine	✓
Proclin	✓
Borate buffer	✓
Nucleophilic components *(Primary amines e.g. amino acids or ethanolamine and thiols e.g. mercaptoethanol or DTT)*	✓

3.6 Purification Example:



SDS-PAGE Gel showing the use of the BSA Removal Kit on a mixture containing 1 mg/mL IgG and 1 mg/mL BSA. The gel shows the mix before and after separation.

3.7 Pellet (antibody precipitates) Example



The pellet in the bottom of 1.5ml Eppendorf type tube showing the use of the BSA Removal Kit on a Ab precipitates of 50µg, 100µg and 500µg.

4. Assay Procedure

4.1 BSA Removal Buffer may contain precipitated material. To dissolve the aggregates, place the vial containing the BSA Removal Buffer in a water bath at 40°C for about 10 minutes until the aggregates have dissolved. You can shake vial to re-dissolve the contents. **Caution!** DO NOT heat above 44°C.

4.2 If the precipitate in the buffer does not dissolve completely. Spin it in a bench top micro-centrifuge, at a recommended maximum speed of 13,000 x *g* for 1 minute, and use the supernatant.

4.3 For every 100 µL of antibody to be treated, add 80 µL of the BSA Removal Buffer directly to the antibody solution.

4.4 Mix and incubate for 5 minutes at room temperature.

4.5 Spin the sample in a microfuge, at a recommended maximum speed of 13,000 x *g* for 5 minutes, until a pellet is formed. Position the tube in the centrifuge in such a manner that you know where any pellet will be located. We routinely position the hinge of the tube at the outside edge of the rotor.

*Δ **Note:** Required spin time will vary depending on buffer composition and speed. The speed should not exceed 13,000 x g.*

4.6 Remove the sample from the centrifuge, taking care not to dislodge the small pellet at the bottom of the tube. Remove the supernatant. The supernatant can be kept on ice until a positive outcome is confirmed.

4.7 Re-suspend the pellet using the Re-suspension Buffer provided, or another buffer suitable for the labeling process.

*Δ **Note:** Required spin time will vary depending on buffer composition and speed. The speed should not exceed 13,000 x g.*

*Δ **Note:** If, after centrifugation, the supernatant appears cloudy and slightly viscous, a precipitate may have formed*

but not have become a pellet. If a pellet cannot be seen, but there was precipitation on addition of the BSA Removal Buffer, add 10% volume of water, incubate for a further 5 minutes, and centrifuge as before. If a pellet can't be seen and no precipitation was observed after addition of the BSA Removal Buffer, add another 10% volume of BSA Removal Buffer and centrifuge again. In the absence of a pellet at this stage, please contact our Technical Support Team before continuing.

5. Antibody Storage

Store at +4°C. Other storage conditions (e.g. frozen at -70°C) may also be satisfactory). The sensitivity of any particular antibody to freeze-thaw should be determined by experimentation on small aliquots.

6. Notes

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)