# ab204912 BSA Removal Kit (Gold/Europium labelling)

# A product of Expedeon, an Abcam company

Applicable to Expedeon product codes: 263-0100

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BSA Removal Kit (Gold/Europium labelling) datasheet:

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For the removal of BSA from antibodies.

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, BSA in the antibody formulation 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 BSA. BSA may also need to be removed prior to other antibody applications. Common commercial BSA removal techniques can involve many laborious steps.

BSA Removal Kit (Gold/Europium labelling) (ab173231) is a simple one-step, 10 minute method that effectively separates BSA from the antibody. Antibodies purified using the BSA Removal Kit (Gold/Europium labelling) are fully compatible with our Gold, Magnetic, Latex and Europium conjugation kits (available separately). The BSA Removal Kit can be used on any antibody subtype, and species.

# 2. Materials Supplied and Storage

Store kit at +4°C immediately on receipt. Do not freeze or store the resin at room temperature. Freezing the suspension will damage the agarose beads.

Item	Quantity	Storage Condition
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 Antibody/BSA Concentration

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

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-ionised, distilled water until the BSA concentration is 0.5% or less.

## 3.2 Buffer Composition

Buffers such as MES, Tris and PBS are compatible with the kit. 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 deionised water until the glycerol content is 20% or less. This protocol can then be followed as written.

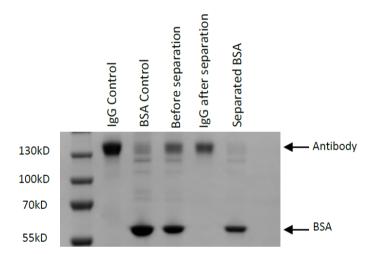
The BSA Removal kit is effective with buffers between pH 6.0 and pH 8.0. If the buffer is outside the suggested pH range, please contact our Technical Support team.

The kit can also be used to concentrate your antibody.

# 3.3 Compatibility with Nanoparticle Conjugation Kits

Antibodies purified using the BSA Removal Kit (Gold/Europium labelling) are fully compatible with our Gold, Latex, Europium and Magnetic particle conjugation kits, providing the antibody is purified and resuspended at a sufficient concentration for the conjugation reaction. We recommend a stock concentration of purified antibody of 1 mg/mL. For more information on required concentrations, consult the protocol for the applicable conjugation kit.

#### 3.4 Example Purification:



**Figure 1.** The image is a non-reducing SDS-PAGE Gel showing the use of the BSA Removal Kit on a mixture containing 1 mg/mL IgG and 3 mg/mL BSA. The gel shows the mix before and after separation.

# 3.5 Contaminating products

The BSA Removal kit can also be used to remove the following low molecular weight contaminants from your antibody buffer:

Buffer components	Removal
Non-buffering salts (e.g. NaCl)	Yes
Chelating agents (e.g. EDTA)	Yes
Sugars	Yes
Glycerol	<20%
Thimerosal / Thiomersal	Yes
Merthiolate	Yes
Sodium Azide	Yes
BSA	Yes
Gelatin	No
Tris	Yes
Glycine	Yes
Proclin	Yes
Borate buffer	Yes
Nucleophilic components (Primary amines e.g. amino acids or ethanolamine and thiols e.g. mercaptoethanol or DTT)	Yes

# 4. Assay Procedure

- 4.1 BSA Removal Buffer may contain precipitated matter. To dissolve this matter, place the vial containing BSA Removal Buffer in a water bath at 40°C for 10-15 minutes until the aggregates have dissolved. DO NOT heat above 44°C. Once dissolved, maintain the tube at ~22°C to prevent any crystal formation before use.
- 4.2 If the sample does not dissolve completely, spin it in a bench top micro-centrifuge, at a recommended maximum speed of 13,000 g for 1 minute, and use the supernatant.
- **4.3** For every 100 μL of antibody to be treated, add 80 μL of 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\*
- **4.6** 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 labelling process.

#### A Notes:

\*Required spin time will vary depending on buffer composition and speed.

\*\*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 antibody to freeze thaw should be determined by experimentation on small aliquots.

#### 6. FAQs

#### 1. How much BSA can the BSA Removal Kit remove?

This 1 ml kit can remove all of the BSA from up to 1.25 mL of antibody, with a BSA concentration of 0.5% or less. For higher BSA concentrations, the method may need to be repeated, or a higher volume of BSA Removal Buffer may be required.

### 2. Can the BSA Removal Kit remove gelatin from my sample?

No, the kit is specifically designed for the removal of BSA. It is effective on some other buffers (see question 4 and section 3.5), but is not effective on gelatin.

# 3. Could I use the BSA Removal Kit to remove Tris or Glycine from my antibody?

Yes, the kit will effectively separate the antibody in this situation.

## 4. Can the kit be used to purify antibody from TCS or serum?

No, the kit is not specific enough to the antibody to be used as a purification technique in this instance.

## 5. Can the BSA Removal Kit be used to concentrate a sample?

Yes, once the separation is complete, the antibody pellet can be recovered using any volume, to reach the desired final concentration.

# 6. Could the kit have any negative impact on the Gold conjugation efficiency?

No, the BSA Removal Buffer has no effect on antibody conjugation using Gold kits.

# **Technical Support**

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