ab102778 – Antibody Concentration Kit Protocol

For preparing antibodies for conjugation

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

Version 1 Last Updated 28 October 2013
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1. Introduction

Antibodies are sometimes only available at low concentrations and often contain low molecular weight substances that interfere in labeling reactions with enzymes, biotin, streptavidin and fluorophores. The Antibody Concentration Kit allows for the quick and easy concentration of antibodies and proteins. The kit can also be used to reduce the concentration of many unwanted additives often found in antibody formulations such as azide, glycine or tris.

The Antibody Concentration Kit method utilizes a simple spin column to easily and quickly remove excess buffer from the antibody thereby providing a more concentrated antibody solution. The Antibody Concentration Kit also allows the experimenter to perform a simple buffer exchange to transfer the antibody.

The components of Ab102778 are fully compatible with our Antibody conjugation kits.
2. Kit Contents

<table>
<thead>
<tr>
<th></th>
<th>1 x Test</th>
<th>3 x Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conjugation Buffer</td>
<td>1 vial</td>
<td>3 vials</td>
</tr>
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</table>

3. Reagents Required, Not supplied

- Antibody to be concentrated

4. Storage and Handling

Kits are shipped at ambient temperature in a tamper-evident polypropylene container. Store at 4°C upon receipt.
5. Concentration Protocol

1. **Concentration of Antibody Solution**

   Add antibody to spin cartridge.

   Spin for 1 to 3 minutes* in a microfuge at max speed of 15000g to reduce the buffer volume in the spin cartridge to between 50 and 100ul. It is advisable not to spin the antibody dry as reconstitution of the antibody will be difficult and significant antibody loss and degradation may occur.

   Repeat steps 1 to 2 as many times as is necessary to process the entire antibody to the desired concentration. It may be necessary to discard the excess buffer collected in the collection tube between spins.

   Recover the concentrated antibody from the spin cartridge.

   *NB. It is advisable not spin the antibody dry as reconstitution of the antibody will be difficult and significant antibody loss and degradation may occur.*

2. **Buffer Exchange Using Spin Column Assembly**

   Add up to 0.5ml antibody to spin cartridge.
Spin for 1 to 3 minutes* in a microfuge at maximum speed to reduce the buffer volume to 100ul.

Discard the excess liquid in collection tube.

Add 400ul conjugation buffer to the antibody in the spin cartridge.

Spin for 1 to 3 minutes* in a microfuge at maximum speed to reduce buffer volume to 100ul.

Discard the excess liquid in collection tube.

Repeat steps 1 to 6 at least 5 times to exchange antibody buffer.

Recover antibody from the spin cartridge.

**Note** Each cycle leads to a reduction in the concentration of low molecular substances. By performing as many as 5 repeat steps the concentration of small molecules such as glycine and Tris will be reduced 2500 fold. However, the concentration of proteins such as BSA will be unchanged.

To remove unwanted proteins see ab102784.
6. Antibody pre-conjugation considerations

The purified antibody to be labeled should ideally be in 10-50mM amine-free buffer (e.g. MES, MOPS, HEPES, PBS), pH range 6.5 to 8.5. If the buffer is more concentrated or outside this pH range contact the technical support team.

Common non-buffering salts (e.g. sodium chloride), chelating agents (e.g. EDTA), and sugars have no effect on conjugation efficiency. Azide (0.02 to 0.1%) and BSA (0.1 to 0.5%) have little or no effect. Glycerol up to 50% has no effect.

Avoid buffer components that are nucleophilic, as these may react with chemicals. Primary amines (e.g. amino acids or ethanolamine) and thiols (e.g. mercaptoethanol or DTT) fall within this class (Note: Tris-based buffers should be avoided). If your buffer contains primary amines and/or thiols, you should consider using our Concentration and Purification Kits.
7. Storage of Antibody

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.
8. General FAQs

1. **What is the optimal starting concentration for the antibody?**

   The antibody conjugation kit allows antibody labeling to be performed on a microgram to milligram scale. The amount of antibody should correspond to molar ratios between 1:4 and 1:1 of antibody to conjugate. Based on their molecular weights (e.g. 160kDa for the antibody versus 40kDa for the conjugate), 100-400μg of conjugate can be added to 100μg of antibody. Antibody concentrations of 0.5-5mg/ml give optimal results. We recommend using 10μl, 100μl and 1ml of antibody solution with the 10μg, 100μg and 1mg kit formats, respectively. The antibody concentration for each conjugation kit has been optimised. Please refer to the relevant datasheet or protocol for the recommended antibody concentration.

2. **Do I need to purify the antibody before using the conjugation kit?**

   Yes. The antibody labeling chemistry involves free amine groups. Most proteins/peptides have lysine and/or alpha-amino groups, therefore, any protein/peptide present in the solution will also be labeled. We recommend purifying your antibodies before performing the conjugation. Ascites fluid, serum or hybridoma
culture media should be avoided. View compatible and incompatible buffers in question 5.

3. **Are conjugation kits suitable for proteins and secondary antibodies?**

Yes. The labeling chemistry involves free amines present in lysines and at the N-terminus of a protein. All antibodies have multiple free amine groups and most proteins have lysine and/or alpha-amino groups. As long as lysines are present, secondary antibodies and proteins will be labeled with the conjugation kits. However, they have not yet been specifically tested with secondary antibodies and proteins.

4. **What buffers can be used?**

We recommend using Hepes, MES, MOPS and phosphate-based buffers or any other amine-free buffer. Conjugation reactions can also be prepared in the presence of up to 20mM Tris buffer with almost no reduction in coupling efficiency. Once the reaction is complete, the conjugated antibody can be diluted in any buffer compatible with both label and antibody.

5. **Which buffer additives can be used and what should be avoided?**

Additives such as salts (e.g. NaCl), sugars (e.g. sucrose) and chelators (e.g. EDTA) have no effect on the labeling reaction. We
recommend avoiding nucleophiles such as amino acids (e.g. glycine), blockers (e.g. ethanolamine) and thiols (DTT, mercaptoethanol) that might deactivate the chemical which covalently links the conjugate to the antibody.

**Compatible additives:**

- up to 20mM Tris
- up to 0.5% BSA
- up to 0.1% gelatin
- up to 0.1% sodium azide
- up to 0.1% sodium azide
- PBS pH7.4
- up to 0.1% sodium azide
- Proclin 300
- up to 50% glycerol
- 0.02M potassium phosphate
- 0.001% Tween
- 5% Trehalose
- 0.015M sodium chloride
- 50mM HEPES

**Incompatible additives:**

- 60mM citrate + 150mM Tris pH7.8
- Ethanolamine
- Urea
- DTT
- 50mM Imidazole
- Mercaptoethanol
- Glycine
6. **How do I remove additives from the antibody storage buffer?**

Our Antibody Concentration and Purification kits remove additives with ease and provide a ready-to-use antibody solution compatible with the conjugation kit.

The Antibody Concentration kit allows an easy concentration and reduction of azide, glycine and Tris.

The Antibody Purification Kit quickly removes BSA, glycine, Tris, azide etc. and can also be used to purify antibodies from ascites fluid or immune serum.

For further FAQs visit: www.abcam.com/conjugationFAQS
9. Publications using Conjugation kits

Label: Cy3® Application: FRET
Effects of pH on molecular mechanisms of chitosane-integrin interactions and resulting tight-junction disruptions

Label: R-PE Application: Immunocytochemistry and In-vivo imaging*
Intraoperative Imaging of Metastatic Lymph Nodes Using a Fluorophore-conjugated Antibody in a HER2/neu-expressing Orthotopic Breast Cancer Mouse Model

*N.B. No product warranty for this application

Label: APC/Cy7® Application: Flow cytometry
APR-246/PRIMA-1M is rescued epidermal differentiation in skin keratinocytes derived from EEC syndrome patients with p63 mutations

Label: FITC Application: Flow cytometry and cellular assay
Constitutive dimerization of glycoprotein VI (GPVI) in resting platelets is essential for binding to collagen and activation in flowing blood.

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