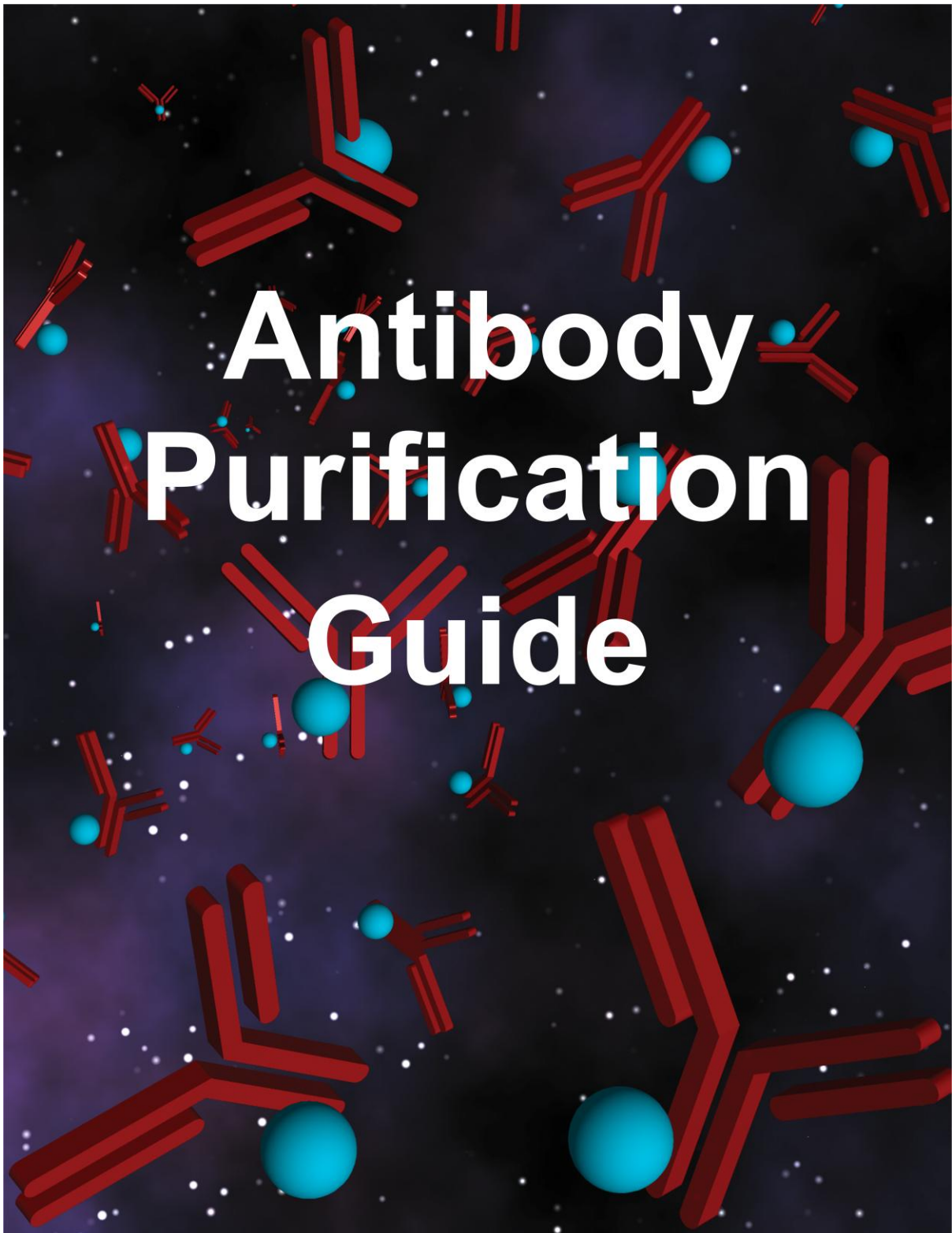




Innova Biosciences
first for Antibody labeling



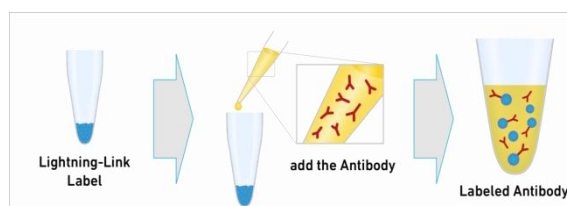
Lightning-Link – Simple Antibody Labeling Kits

The simplification of the [antibody labeling](#) process (most notably the elimination of column separation steps) circumvents many issues that have beset traditional procedures for years -loss of material, sample dilution during column chromatography, batch-to-batch variation and difficulties in scaling up.

The Lightning-Link process is summarized in the figure below. You simply pipette the antibody to be labeled into a lyophilized mixture containing the label of interest. Dissolution of the vial contents activates the chemicals that mediate the antibody labeling reaction. As there are no purification or separation steps (byproducts of the reaction are completely benign), antibody recovery is close to 100%. Furthermore the labeling reaction can be set up using this simple method in less than thirty seconds. You can see a timed demonstration ([Lightning-Link video](#)) of the antibody labeling process.

Go Direct!

With Lightning-Link technology you are now able to label any antibody with any one of over 45 labels to generate novel antibody research tools for your pioneering research. Direct labeling of antibodies greatly simplifies immuno-experiments; without the problems of crossover and/or non specific binding from secondary antibodies it is far easier to obtain high quality data. A reduction in the number of tedious incubation and wash steps also saves time and money. Moreover you can now label as little as 10ug of antibody and scale up with ease.



Buffers and Additives – Key Considerations for Antibody Labeling

It is important to remember that your antibody will contain substances other than antibody; minimally a buffer, and possibly other proteins and additives. Compatibility of the mixture with labeling methods may not have been a key consideration when the antibody was initially purified and formulated. Occasionally it will be necessary to re-purify the antibody prior to carrying out the labeling reaction. Purification may involve the removal of stabilizing proteins (e.g. BSA) or low molecular weight substances, such as sodium azide, tris buffer or glycine. Different labeling methods may be negatively impacted to various extents by these additives. As the majority of labeling methods exploit the lysine residues present on antibodies, substances with primary amines should generally be avoided. For example, glycine is often used to elute antibodies from antigen affinity columns and antibodies that have been purified in this way should be extensively dialysed before use in any labeling reactions. Dialysis to remove unwanted low molecular weight substances is more efficient if the buffer is changed two or three times. This does not mean that you have to make three times as much buffer. However it does mean that the total time required for the dialysis procedure will be greater. For example, calculation shows that dialysis against 3 x 1L volumes is far more effective than dialysis against a single 5L volume. PBS, MES or bicarbonate are often recommended as suitable buffers in which to carry out conjugation reactions the choice of which buffer to use is determined by the pH requirements of the labeling reaction.

Antibody Concentration and Purity

For most labeling reactions, the antibody will need to be reasonably pure (e.g. >90%, preferably >95%) and at a concentration of >0.5mg/ml. Many commercially available antibodies are not always provided in a form suitable for labeling. For example, antibodies may be sold in the form of hybridoma tissue culture supernatant (TCS), ascites fluid or crude serum. TCS often contains many

other proteins and culture nutrients (e.g. amino acids) which are particularly problematic. Purification (e.g. on protein A columns) is obligatory if TCS is your starting point. Ascites fluids and crude serum generally have higher concentrations of antibody than TCS, but again these formulations contain many substances that interfere with antibody labeling. Purification of these antibodies will be required.

Accessory Antibody Purification Systems

There are a significant number of antibodies available from commercial suppliers who may or may not provide the antibody in a favorable buffer formulation for antibody labeling. It can often be difficult to identify the best route to take to create an optimal buffer formulation for antibody labeling.

To overcome this problem we have designed a range of accessory kits which can be used to separate antibodies from unfavorable starting points. These kits are extremely easy and convenient to use and are fully compatible with the Lightning-Link range. Combining an accessory kit with a Lightning-Link product a user is now in the unique situation to label an antibody irrespective of the antibody starting formulation.

Each kit is briefly described below:

The AbSelect range involves the capture of the antibody on a protein A resin that has been immobilised to agarose beads and can be used to purify IgG fractions. Unwanted substances are easily removed by incorporating simple wash steps, and the captured antibody is subsequently eluted.

[AbSelect™ Antibody Purification System](#) (860-0010) readily removes unwanted substances such as proteins, preservatives, amino acids, and other components that can interfere with labeling reactions. It can either be used to purify antibodies from crude samples - (ascites fluid or serum) or poor buffer formulations (buffers containing high concentrations of BSA, azide or Tris). The antibody to be purified or cleaned up ideally is up to 0.5mg and in a volume of 100 to 500ul

[AbSelect Serum Purification Systems](#) (863-0500) antibodies generated from ascites fluid and serums are often supplied as crude formulations. The AbSelect Serum system is a fast and simple method to purify antibodies from these types of media and can be used to purify up to 20mg of antibody.

[AbSelect TCS System](#) (862-0500) - Antibodies are often generated from hybridoma cell lines and supplied in tissue culture supernatant (TCS). The AbSelect TCS system is a fast and simple method to purify antibodies from 10-50mls of TCS.

[Antibody Concentration and Cleanup Kit](#) (861-0010) - allows for the quick and easy concentration of antibodies. The kit can also be used to reduce the concentration of any undesirable low molecular weight additives. The antibody clean up kit method utilizes a simple spin column to easily and quickly remove excess buffer from the antibody thereby providing a more concentrated antibody solution.

To assist in choosing the best accessory kit the following decision diagram was devised. Please note – the diagram is based upon the most common buffer formulations found in the commercial market place. For formulations which are not depicted within the diagram, we suggest making contact with Innova's technical department (info@innovabiosciences.com) for advice and recommendations.

