

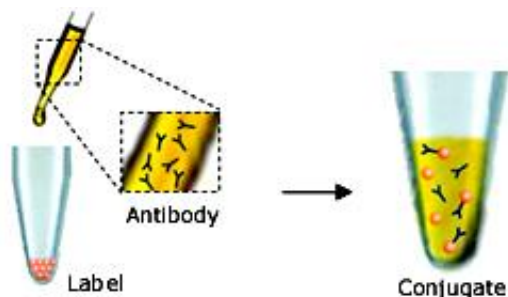


Lightning-Link™ conjugations in the presence of BSA

1. INTRODUCTION

Lightning-Link™ technology reduces the hands-on time for production of antibody (protein) conjugates to less than 30 seconds. The antibody to be labelled (in an amine-free buffer) is simply added to a tube of Lightning-Link™ mix. Since there are no other steps the recovery is essentially 100%.

Fig 1. One-step™ conjugation method



Many commercially available ‘purified’ antibodies are formulated with BSA as a stabilizer. As attempts to remove BSA from small quantities of antibody (e.g. 100µg pack size) inevitably lead to substantial losses of antibody, it is widely felt that the presence of BSA precludes the labelling of many antibodies that are available to researchers. However, the conditions used in Lightning-Link™ conjugation reactions mean that good quality conjugates can be prepared in the presence of surprisingly high concentrations of BSA.

2. EXPERIMENTAL

Goat anti-rabbit IgG (1mg/ml) in PBS was conjugated to HRP in the presence of varying amounts of BSA using a Lightning-Link™ HRP conjugation kit (Product code: 701-0000) according to the instructions.

The efficiency of conjugation was determined by testing dilutions of the resulting anti-rabbit HRP conjugates on a rabbit IgG-coated 96-well plate. Plate coating, blocking and antibody incubations were carried out using standard ELISA methodology.

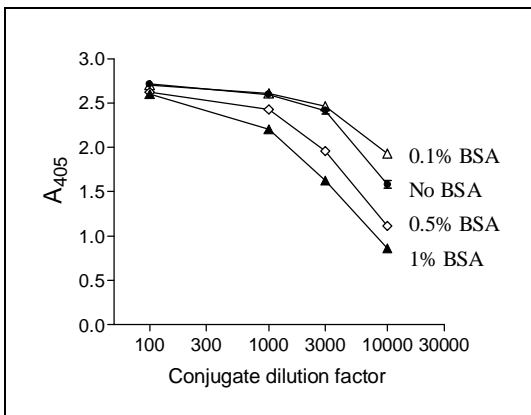
Bound HRP conjugate was detected by incubating with 50µl of 1mM ABTS substrate in sodium acetate pH 5.0 in the presence of H₂O₂ for 10 minutes at room temperature. The absorbance was measured at 405nm.

3. RESULTS

Fig 2 (below) shows the effect of BSA at four concentrations 0%, 0.1%, 0.5% & 1% (i.e. 0 mg/ml, 1 mg/ml, 5 mg/ml & 10 mg/ml) on conjugation of Goat anti-rabbit IgG (1mg/ml) using Lightning-Link™ HRP.

As can be seen, when the concentration of BSA is 0.1% (1 mg/ml) the resulting conjugate is as good as, and probably slightly better than, the conjugate prepared in the absence of BSA.

Fig 2. ELISA to show effect of BSA on conjugation performance



In a more extreme case (1% BSA, i.e. 10 mg/ml BSA and 1 mg/ml antibody) the reduction in performance is far less than one might expect given the substantial excess of BSA. The absorbance at 1/10,000 dilution of conjugate prepared in the presence of 1% BSA (>20-fold molar excess) is reduced only by a factor of 2. The absorbance can be returned to the original 'BSA-free' value simply by diluting the conjugate 1/3,000 instead of 1/10,000.

4. CONCLUSION

At concentrations of BSA found in some antibody preparations, and under the conditions employed in Lightning-Link™ reactions, BSA has only a modest effect on conjugation efficiency. In some cases a slight enhancement of performance is seen.

While these findings are somewhat counterintuitive (Lightning-Link™ requires the presence of amines on the molecule to be labelled) the special conditions employed in Lightning-Link™ reactions allow good quality conjugates to be prepared even in the presence of BSA.

The advantages of directly labelled antibodies are many, including elimination of secondary reagents, lower backgrounds, fewer washes, less hands-on time, and improved data quality.

For further information and Tech Notes on Lightning-Link™ technology please call 44(0)1223 496170 or check our website: www.innovabiosciences.com.