



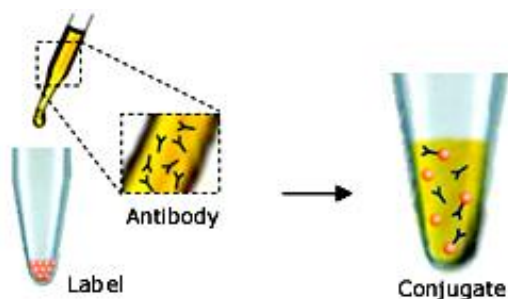
### Lightning-Link™ conjugations in the presence of Gelatin

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#### 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 gelatin as a stabilizer. As attempts to remove gelatin 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 gelatin 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 gelatin.

#### 2. EXPERIMENTAL

Goat anti-rabbit IgG (1mg/ml) in PBS was conjugated to HRP in the presence of varying amounts of gelatin 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.

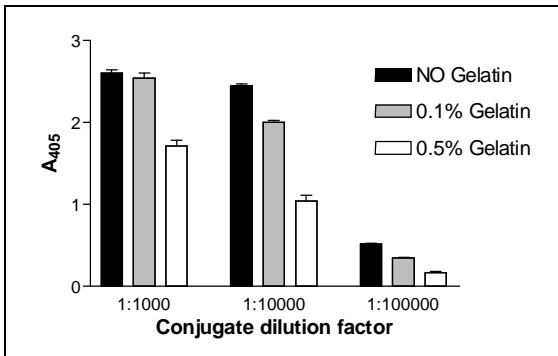
Bound HRP conjugate was detected by incubating with 50µl of 1mM ABTS substrate in sodium acetate pH 5.0 in the presence of H<sub>2</sub>O<sub>2</sub> for 10 minutes at room temperature. The absorbance was measured at 405nm.

#### 3. RESULTS

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

As can be seen, when the concentration of gelatine is 0.1% (a common commercial formulation) the resulting conjugate at 1:10000 dilution is still good retaining >80% of the activity seen with the conjugate prepared in the absence of gelatin.

**Fig 2. ELISA to show the effect of gelatin on conjugation performance**



Moreover, even in an extreme case (0.5% gelatin) the resulting conjugate at 1:10000 dilution is still good retaining around half of the activity seen with the conjugate prepared in the absence of gelatin.

#### **4. CONCLUSION**

At concentrations of gelatin found in some commercial antibody preparations, and under the conditions employed in Lightning-Link™ reactions, gelatin has only a modest effect on conjugation efficiency.

While gelatine interferes in many conjugation methods, the special conditions employed in our Lightning-Link™ reactions allows good quality conjugates to be prepared even in the presence of high concentrations of gelatine.

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](http://www.innovabiosciences.com).