

Technical Note

Anthraquinone chemistry



By Kenneth Harlow and Henrik M. Pfundheller

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Protocol for Coupling Proteins to Exiqon's Immobilizer™ Reagent

Background:

The proprietary Immobilizer™ reagent, see figure 1, incorporates an electrophilic functional group that will react with any good nucleophile.

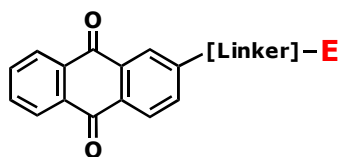


Figure 1. Exiqon's proprietary AQ-Immobilizer™ reagent. The electrophilic group, E, is separated from the photoreactive anthraquinone via a ethylene glycol linker.

In the case of proteins, this essentially limits reaction to primary amine and thiol groups, see figure 2. Other potential nucleophilic groups on proteins such as tyrosine hydroxyl and histidine imidazole groups are either not nucleophilic at the pH's where most

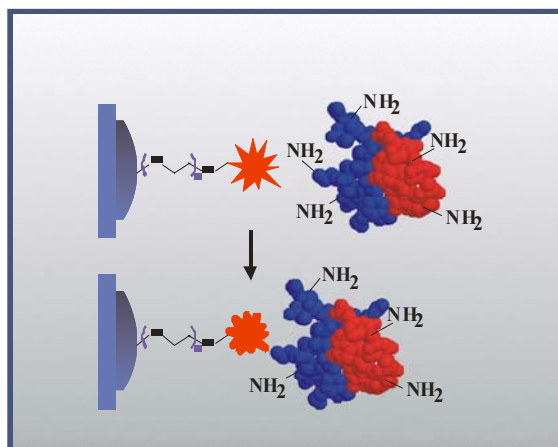


Figure 2. Coupling of protein to surface bound AQ-Immobilizer™ reagent.

proteins are stable, or are such weak nucleophiles that they are competed out by both the overabundance and higher reactivity of the highly nucleophilic amine and thiol groups. Furthermore, reactivity of the amine and thiol groups is a function of pK_a and can be modulated by pH. Thus, amines are poor nucleophiles when they are protonated and are expected to react less vigorously at pH's below the pK_a of the ϵ -amine group, whereas the thiol group

of cysteine reacts more vigorously in the thiolate form found above the pK_a of the thiol group.

Therefore, it may be possible to limit reaction to thiol groups by running reactions closer to neutral pH where the thiolate anion will be the most reactive species. These considerations must be weighed with care however, as the pK_a 's of ionizable groups in proteins are often very different from the pK_a 's of the respective amino acid side chains in solution and therefore may not reflect the reactivity of the potential nucleophilicity of these groups in proteins.

Suggested Guidelines & Protocol:

The chemistry of proteins is very diverse and it is therefore difficult to develop a generalized protocol for coupling with the Immobilizer™ reagent™ that works well for all proteins. A certain amount of optimizing may be necessary to obtain the best results. Since the target nucleophiles are either primary amines or thiols, the Proteomics group at Exiqon has employed two different conditions for the covalent coupling reactions. It is our experience that one of these conditions will usually provide at least acceptable results, but users are encouraged to take into consideration the individual properties of the proteins with which they are working and develop their own set of conditions if need be.

- Proteins are coupled to the Immobilizer™ reagent using two different buffer systems:
 - Phosphate buffered saline (PBS; 10 mM Na phosphate buffer, pH 7.5, 150 mM NaCl)
 - 100 mM Na carbonate, pH 9.6

PBS will favor coupling thiols alone, whereas carbonate will facilitate reaction with both amine and thiol functionalities.

- When coupling proteins to surfaces coated with the Immobilizer™ reagent, it is suggested that a dilution series of the protein of interest be run in the two buffers above. A suitable protein concentration at which to start is around 100 $\mu\text{g/ml}$. Coupling times vary and should be determined empirically, but a good starting place is a 1 hour incubation at ambient temperature. Be aware that both temperature and protein concentrations affect the reaction rate. Furthermore, amine buffers and other nucleophiles should not be present in the protein solutions used for immobilization.
- After coupling to the surface, remaining Immobilizer™ electrophilic groups are quenched by reaction with 10 mM ethanolamine in 100 mM Na carbonate, pH 9.6 buffer for 1 hour at ambient temperature. This eliminates the possibility of

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the surface reacting at a later point in time with other non-relevant nucleophiles and also introduces an hydroxyl functionality which makes the surface more hydrophilic and less prone to non-specific adsorption. The surface can now be used for immunoassay purposes without the need for a non-relevant blocking protein to be present in assay buffers.

Additional information:

More information concerning immobilization of proteins onto the Immobilizer™ reagent can be found at [www.exiqon.com/library/technical notes/Immobilizer Series/ Protein Immobilizer™](http://www.exiqon.com/library/technical%20notes/Immobilizer%20Series/Protein%20Immobilizer™).

Trademarks and patents

The anthraquinone technology is covered by U.S. Patent no. 6,033,784, EP 0820483 (Nationally filed in Albania, Austria, Belgium, Finland, France, Germany, Greece, Ireland, Italy, Latvia, Lithuania, Luxembourg, Monaco, Netherlands, Portugal, Slovenia, Spain, Sweden, Switzerland and United Kingdom), JP 3124037 and AU 699321 owned by Exiqon A/S.

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