Inject and elute injection command using Biacore™ 1S+ and Biacore 1K+ SPR systems

This procedure describes how the ***Inject and elute*** command is used in Biacore™ 1S+ and Biacore 1K+ instruments. The command is used to elute and collect bound analyte from the ligand on the sensor surface for downstream analysis. The ***Inject and elute*** command can thus be used to "fish out" an analyte that binds a specific target on the sensor chip from a complex sample solution, e.g., tissue and plant extracts. To maximize the amount of collected analyte, the ***Inject and elute*** command is designed to address all six flow cells of the flow system in series. Thus, the command is only available when all six flow cells are selected.

Change flow path settings in the ***General settings*** panel in the method before adding an ***Inject and elute*** command (Fig 1). Click ***Change flow cells*** and change the flow cells to include all flow cells, i.e., 1, 2, 3, 4, 5, 6. The ***Inject and elute*** command is only available for instruments that address six flow cells in series, i.e., Biacore 1K+ and Biacore 1S+. The general principle for the ***Inject and elute*** command is the same as for the ***Inject and recover*** command in Biacore T200 and Biacore 3000.

Assay solutions – description

- Deposition solution: Deposition solution is used to neutralize the eluent solution, which is often acidic or basic. A minimum volume of 10 µL is necessary to collect the recovered sample. The deposition solution is automatically delivered by the instrument to a target position in a microplate or a vial in the reagent rack. The volume of the deposition solution is a variable and is determined in the control software by the user.
- Analyte solution: A sample solution that contains the active substance and binds to the immobilized ligand.
- Wash solution: A solution used to clean the flow system from sample components that may have bound non-specifically to tubings and parts of the flow system. The wash solution does not pass over the flow cell.
- Eluent solution: A solution to dissociate bound sample from ligand on the surface. The eluent solution is injected over the ligand and is then stopped for a specified incubation time, to allow bound analyte to dissociate into the eluent solution. The incubation time can be varied in control software by the user, range 20–1800 s. After the incubation period has passed, the eluent is transferred to the target position.

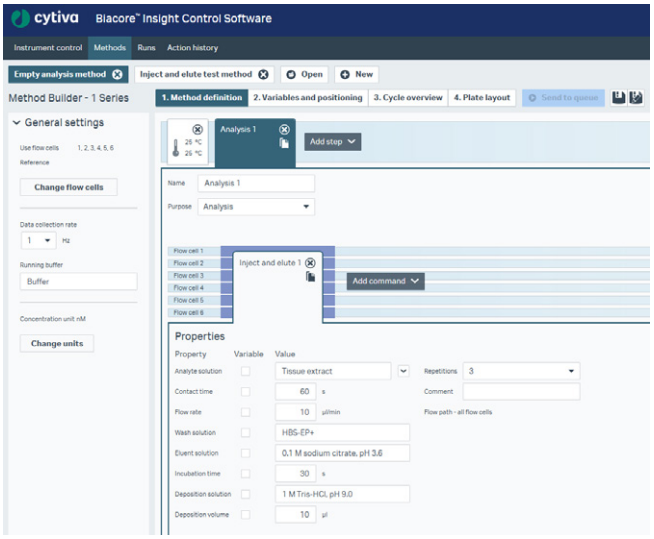

Fig 1. ***General settings*** window in Biacore Insight Control Software.

Assay repetitions

The primary goal of ***Inject and elute*** is to collect analyte with a concentration high enough to enable subsequent analysis. By performing repetitive elution and deposition cycles into the same target position, the final volume is kept low and analyte concentration is increased. The software allows up to 10 repetitions, default is set to three repetitions but can be changed by the user. The lowest deposition solution volume is 10 µL.

Considerations for choosing assay solutions

The choice of deposition solution, wash solution, and eluent solution is assay-specific. Overall, the choice of eluent solution is similar to the choice of regeneration solution but should be less harsh to preserve binding activity of the eluted sample. The solutions also need to be compatible with the Biacore system. In general, the flow system components withstand long-term exposure of common aqueous buffer solutions used in biochemical laboratories. However, concentrated organic solvents as well as long-term, i.e. > 10 min, exposure to extremes of pH (< 3 and > 11s) should be avoided.

More information can be found under chemical resistance in the Biacore 1 series operating instructions. For recommendations concerning substances not listed, please contact your Cytiva representative.

A suitable starting point for selecting assay solutions for ***Inject and elute*** is often to choose conditions similar to what would have been used for affinity purification. An important consideration is also to select the mildest possible eluent conditions if the intention is to maintain binding activity of the recovered sample. An extended incubation time may improve the elution, if it is difficult to dissociate the sample under mild conditions. However, an extended incubation time entails a risk that the binding activity is not preserved. The conditions on the sensor surface are miniaturized compared to an affinity column, and a somewhat lower ionic strength often works compared to what would be used in affinity purification, e.g., 10 mM glycine-HCl instead of 100 mM glycine-HCl.

The most widely used elution buffer for affinity purification based on protein interactions is 0.1 M glycine-HCl, pH 2.5– 3.0. This buffer effectively dissociates most protein-protein and antibody-antigen binding interactions without permanently affecting protein structure. However, some antibodies and proteins are damaged by low pH, so eluted protein fractions are best neutralized immediately by addition of 1:10 volume of alkaline buffer such as 1 M Tris-HCl, pH 8.5. Other elution buffers for affinity purification of proteins are listed in Table 1.

Common elution solutions for Biacore systems

The conditions in Table 1 primarily apply to protein-protein binding interactions, such as between an antibody and its peptide antigen. Elution solutions for binding interactions between other kinds of molecules may be different. Some of the solutions listed below may harm proteins. Consider starting with the mildest possible condition e.g., pH 3.0 or higher.

Table 1. Common elution conditions for protein-protein interactions

Condition	Solution
pH	10 mM glycine-HCl, pH 3.0* 10 mM glycine-HCl, pH 2.5* (< 10 min) 10 to50 mM citric acid, pH 3.0 0.1 M NaOH, pH 13.0 (< 3 min)
Ionic strength and/or chaotropic effect	3.0 M magnesium chloride*† (< 3 min) 0.5 to1 M NaCl 5 M NaCl (< 3 min)
Denaturing	2 to 6 M guanidine-HCl (< 1 min) 2 to 8 M urea (< 1 min) 0.02% to 0.5% SDS‡
Organic	50% ethylene glycol (< 10 min) 100% ethylene glycol (< 1 min) Formic acid up to 70%§ (< 3 min) 0.2% to 1.0% (v/v) trifluoroacetic acid (TFA)

*Ready-to-use solution provided by Cytiva.

† $MgCl_2$ can cause precipitation with phosphate buffer, so avoid using running buffer containing phosphate.

‡ SDS may precipitate if the potassium content of the running buffer is too high.

§ 70% formic acid may dissolve to some degree in the IFC channel walls and leach out in subsequent injections. If formic acid is used for elution, be sure to wash the system until the baseline is stable before performing the next injection.

Small molecules

Small molecules are generally more tolerant of harsh conditions than macromolecules, so that a wider range of elution conditions can often be considered. The condition in Table 2 applies primarily to elution of small molecules.

Table 2. Common elution conditions for protein-small molecule interactions

Condition	Solution
pH	10 mM glycine-HCl, pH 2.0*(< 3 min) 10 mM glycine-HCl, pH 1.5*(< 1 min) 10 to 100 mM HCl (< 1 min)
Organic	Up to 50% acetonitrile (< 1 min) Dimethyl sulfoxide (DMSO) 50% (< 10 min)

*Ready-to-use solution provided by Cytiva.

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