

Degas buffers for use in affinity and gel filtration columns

TR0029.1

Introduction

Air bubbles that are introduced into column resin beds during shipping or in buffers containing dissolved gas may clog or impede the flow of solutions through gravity-flow affinity or gel filtration columns. Dissolved gases in buffers can come out of solution (outgas) while passing through a porous gel matrix, coalescing to form small bubbles within the resin bed. Although they might be too small to visualize, these bubbles will interfere with normal column function. In addition to slowing the flow of buffers through a column, air bubbles prevent solutions from flowing through the entire column, reducing contact with the immobilized ligand and resulting in decreased binding capacity and elution efficiency.

The following protocol is a quick method for removing dissolved air from (degassing) buffers and other solutions before applying them to a column to prevent the introduction of air bubbles into the gel bed. Whenever possible, degas all buffers and large sample volumes before using affinity purification columns or gel filtration (desalting) columns. Although using degassed solutions is seldom necessary with centrifugal or pumped column procedures, it is often a critical factor in success of small-scale gravity-flow methods.

Procedure for Degassing Solutions

1. Transfer buffer to vacuum flask and seal top of flask with rubber stopper.
2. Attach hose to sidearm of vacuum flask, with other side of hose attached to vacuum source.
Note: If vacuum flask is not available, an Erlenmeyer flask and rubber stopper with one hole in the top can be substituted. Non-collapsible tubing must be inserted through the hole in the rubber stopper into the opening in the flask. Attach hose to the side of the tubing that is above the surface of the rubber stopper.
3. Turn on vacuum to low, observing buffer in flask at all times. Bubbles may appear to rise out of the buffer or the buffer may appear to “boil” as gas is drawn out of the buffer. Gently swirl solution to promote release of all dissolved gases.
Note: Buffers containing detergent or proteins may foam upon exposure to the vacuum. Be prepared to break vacuum (step 4) if foam rises up to the vacuum stem. After degassing such solutions, allow foam to dissipate before applying the solution to a column. If detergent is required in a buffer, consider withholding the detergent from the solution formulation until it can be degassed; then gently stir in the required amount of detergent afterward.
4. When no further bubbling or “boiling” occurs, gently break vacuum seal between atmosphere and flask by slowly removing vacuum hose or rubber stopper from the flask.
5. When connection to the vacuum source is completely broken, shut off vacuum source.
Note: If using a house vacuum or a vacuum pump equipped with a shut-off valve between the pump and flask, step 5 may be performed before step 4. However, if sample flask is directly connected to a vacuum pump, do not turn off vacuum pump until after breaking connection to the flask containing the sample, or else a back-flow of foul air and pump oil may enter sample.

The buffer may now be applied to a column without the risk of air bubbles forming in the column. Maintain buffer in degassed condition by avoiding vigorous shaking or excessive mixing, which will reintroduce air into the solution.

Current versions of product instructions are available at www.thermo.com/pierce. For a faxed copy, call 800-874-3723 or contact your local distributor.

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