Column Washing Protocol

1. After expelling contents of the column, remove plunger and place columns on the vacuum manifold. Turn the manifold on. The quicker the washing occurs following use the less likely clogging or rusting occurs. We usually get to them within an hour.

2. (Optional) Run 5ml PBS. Discard any columns that clog.

3. Run 5 ml dH2O 2x. Discard any columns that clog.

4. Run 5 ml 95% alcohol.

5. Leave on the manifold to dry for 30 or more minutes then remove.

6. Mark columns appropriately to denote the number of uses in case it is discovered that a certain number of uses is detrimental. We use a scissor type rat ear punch to notch the top lip of the column. We have gone out 15 uses so far without any detectable problems.

7. Rinse plungers 1x with 70% ethanol and allow to air dry.

Prior to use:

1. Rinse with 3ml 70% ethanol. Once the ethanol has begun to run all the way through, force a little ethanol through the column using the plunger to force out air bubbles.

2. Rinse with 3ml of whatever media your cells will be in.

3. Run sample as normal.

Notes:

- Discard any rusted columns. Rust isn’t typically an issue unless the columns sit for a while without being cleaned, or are not fully dried.

- We use a vacuum manifold that is designed for Qiagen RNA/DNA isolation columns. LS and LD columns should fit perfectly.

- Clogged columns can often be rescued if you take them off the manifold and force 5 ml of water through with the plunger 2-3 times. Clogging is more of an issue when we do negative selection for B or T cells and there are many bound cells in the column.

- Columns can be autoclaved if sterility is an issue. We haven’t tried this.