



Washing Technique for Microtiter Wells

Our experience has shown that the most frequent cause of customer problems in the performance of ELISA is due to plate washing equipment and technique. While the goal of washing is to remove unbound reactants, many technicians fail to understand that the washing step can also dissociate antibody bound reactants and can do so in a highly variable manner. Our experience has shown that automated plate washers and related multi-channel hand-held vacuum aspiration devices give inferior assay performance relative to the manual procedure described below. Some plate washers may leave behind very small but still significant liquid causing variable and higher assay background than can be achieved manually. In an effort to reduce carryover, many washers can be too aggressive in the physics of washing and aspirating and will actually variably dissociate antibody bound analyte resulting in both lower absorbance and higher %CVs. For these reasons we strongly recommend that users use the manual procedure detailed below for optimal assay performance.

Equipment Required: All that is required for equipment is a wash/squirt bottle and low lint absorbent paper. We suggest that the narrow portion of the tip of the squirt bottle be cut off to give the largest possible orifice so that the flow will be generous and gentle. This procedure is best done over a large sink since it can be a little messy.

Step 1. Dumping the liquid from the plate:

Grab the plate from the bottom with the thumb in the middle of one side and the fingers on the other side. If the thumb and fingers slightly overlap the tabs on the ends of the middle strip or strips you will usually be able to avoid having any strips fall out of the strip holder. Holding the plate over the sink, turn the plate upside down just as you rapidly accelerate your arm and hand downward. Abruptly stop your arm causing the liquid to be forced from the strips into the sink. When done properly you should not get any liquid on your fingers or on the outside of the strip wells or plate holder. Repeat the dumping motion a second time.

Step 2. Blotting and tapping the plate:

Immediately blot the upside down plate onto the blotting paper. Move the plate to an unused section of the blotting paper. Firmly tap the plate 3 times over unused areas of the paper. Do not bang the plate too hard in a misguided effort to remove all of the residual liquid, as this may cause variable dissociation due to transferring of unequal amounts of shock energy across the plate.

Step 3. Washing:

Use the squirt bottle to fill all wells starting at the front of the plate and working to the back. Fill to overflowing with the 20 fold diluted wash solution provided with the kit.

→ **Do not** use any other wash buffer formulation as it may negatively impact the performance of the kit.

→ **Do not** worry about overflowing the wells as you will be wiping off the bottom

of the wells before adding substrate.

→ **Do not** allow the wash solution to soak in the wells.

Immediately dump & tap the plate as described in Steps 1 & 2 as soon as the last well is filled.

Repeat the washing procedure 3 more times for a total of 4 washes. With the 2nd & 4th washes start by adding the wash solution from the back to the front of the plate. This insures that the total dwell time of wash solution in the wells will be essentially the same for all wells. Additional wash steps should not be necessary and in fact may dissociate specific bound analyte and actually reduce assay sensitivity.

After the last wash, blot and tap as described in Steps 1 & 2. Let the plate rest upside down for about 20 seconds to drain. Firmly tap the plate again 4 times rotating the plate 180° in your hand between each tap. This rotation ensures that the ends of the plate receive on average the same energy and impact from the banging.

Step 4. Wiping the plate:

Wipe the bottom outside of all wells with clean absorbent paper to remove any wash liquid from the overfilling and tapping. Wells are now ready to have the substrate added to them. Add substrate immediately after washing.

→ **Do not** let the wells dry out or enzymatic activity will be lost.

→ **Do not** add substrate near the sink location where your dumping and banging have taken place since the washing procedure can generate aerosols that could re-contaminate the wells or your substrate.

Watch the video [Washing Technique for Microtiter Plate ELISA Video](#) to get the exact technique.