

## Washing of 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. Most automated plate washers and related multichannel hand-held vacuum aspiration devices will 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 addition, 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 you 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 or lint free 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 messy.

### 1. Dumping the liquid from the plate:

Hold 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 will not get any liquid on your fingers or on the outside of the strip wells or plate holder. Repeat this dumping motion a second time.

### 2. Blotting and banging the plate:

Immediately blot the upside down plate onto the blotting paper. Move the plate to an unused section of the blotting paper and allow to drain upside down for 5-10 seconds. Bang the plate 4 times over unused areas of the paper.

### 3. Washing:

Using the squirt bottle, fill all wells starting at the front of the plate and working to the back. Fill to overflow with the 20X diluted Wash Solution provided with the kit. As an alternative to using a squirt bottle you may introduce the wash solution using a multi-channel pipettor. In this case, add approximately 350 $\mu$ l of wash solution to each well at each of the 4 wash cycles.

**Do not** touch the inside surface of the wells with the pipet tips.  
**Do not** use any other wash buffer formulation as it may negatively impact the performance of the kit.  
**Do not** worry about overfilling 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 for any period of time.

Immediately dump & bang 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 let the plate rest upside down for about 30 seconds to drain. Bang again for 3-4 times rotating the plate 180° in your hand between each bang. This rotation ensures that the ends of the plate receive on average the same energy and impact from the banging. Wipe the bottom outside of all wells with clean absorbent paper to remove any wash liquid. To determine if you have done the washing technique correctly, look into the wells. You should see only a small film of liquid (<1microliter) in the center of each well. If the film is not uniform in terms of area between the wells or if significant liquid remains in the circular edge of the wells you have not banged the plates hard enough.

Wells are now ready to have the substrate added to them. Add substrate immediately and do not let the wells dry out. **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.