

The goal of plate washing is to remove unbound material. Insufficient ELISA plate washing adversely affects the results by yielding high background, reduced sensitivity, and increased variability in immunoassays. On the other hand, additional wash steps beyond the recommendation should not be necessary and may reduce assay sensitivity.

To begin, dilute the Wash Buffer Concentrate (included with the kit) with deionized water to 1X Wash Buffer, as described in the assay protocol. If necessary, remove the plate sealer or covering that was used in the previous step of the assay. Discard waste into an appropriate receptacle and change lint-free tissues frequently.

## Manual Washing with a Pipettor

NOTE: Manual washing requires a multi-channel pipettor and/or repeat pipettor for efficient delivery of 1X Wash Buffer. If you do not have these tools, washing with a squirt bottle is the recommended technique.

Completely aspirate the liquid from all wells by gently lowering an aspiration tip into the bottom of each well. Take care not to scratch the inside of the well. As an alternative to aspirating, the plate can be inverted to remove the liquid. While inverted, firmly blot the plate on lint-free tissue to ensure no liquid remains on the rim of wells prior to flipping upright. Fill the wells with at least 300 µL 1X Wash Buffer. Immediately remove the liquid by aspirating or inversion. Repeat as described in the assay protocol. After the washing procedure, invert the plate one final time and firmly blot on a fresh, clean, lint-free tissue. If necessary, also blot the bottom of the plate and exterior walls of the wells on a fresh, clean lint-free tissue. Proceed directly to the next assay step to prevent wells from drying out.

## Manual Washing with a Squirt Bottle

Completely aspirate the liquid from all wells by gently lowering an aspiration tip into the bottom of each well. Take care not to scratch the inside of the well. As an alternative to aspirating, the plate can be inverted to remove the liquid. While inverted, firmly blot the plate on lint-free tissue to ensure no liquid remains on the rim of wells prior to flipping upright. Fill a squirt bottle with 1X Wash Buffer. We recommend cutting off the narrow portion of the tip of the squirt bottle, so that the flow will be generous and gentle. This procedure is best done over a sink. Completely fill all the wells with 1X Wash Buffer from the squirt bottle. Immediately remove the liquid by aspirating or inversion. Repeat as described in the assay protocol. After the washing procedure, invert the plate one final time and firmly blot on a fresh, clean, lint-free tissue. If necessary, also blot the bottom of the plate and exterior walls of the wells on a fresh, clean lint-free tissue. Proceed directly to the next assay step to prevent wells from drying out.







## Handheld Washing Manifold

Completely aspirate the liquid from all wells by gently lowering an aspiration tip into the bottom of each well. Take care not to scratch the inside of the well. As an alternative to aspirating, the plate can be inverted to remove the liquid. While inverted, firmly blot the plate on a lint-free tissue to ensure no liquid remains on the rim of wells prior to flipping back upright. Fill a clean polyurethane bottle with 1X Wash Buffer and attach the appropriate wash dispenser. Prime the dispenser until all bubbles have been removed. Using the prepared wash bottle, fill all wells completely with 1X Wash Buffer. Immediately remove the liquid by aspirating or inversion. Repeat as described in the assay protocol. After the washing procedure, invert the plate one final time and firmly blot on a fresh, clean, lint-free tissue. If necessary, also blot the bottom of the plate and exterior walls of the wells on a fresh, clean lint-free tissue. Proceed directly to the next assay step to prevent wells from drying out.

## Automated Plate Washer

If using an automated washer, follow the manufacturer's instructions for plate washing. Once washing is complete, proceed directly to the next assay step to prevent wells from drying out.



