Washing Guide

Small problems with washing can significantly affect results.

Signs of incorrect washing include **edge effects**, **high background**, and **positive/negative results** which are **not confirmed** upon re-testing.

General recommendations

- **Before washing, be sure to verify the absence of crystals in the 20X or 1X solutions.** To dissolve crystals, put the 20X wash solution bottle in very hot water – crystals will dissolve in less than a minute. The wash solution may be stored at room temperature to avoid crystal formation.
- Prepare the wash solution according to the package insert.
- Follow the specific recommendations in your package insert for the number of washes to use at each step of an assay. Most assays require at least 300–350 µL per well per wash. Be careful to fill the wells above the level of the reagents. Do not allow wells to overflow. If this occurs, the test results may be invalid.
- IDvet produces only one type of wash solution. If necessary, wash solution from the same batch may be shared between IDvet kits. Wash solution from other manufacturers will also work with IDvet kits, as will PBS-Tween solution.
- Avoid prolonged soak times unless specifically recommended in the package insert.
- Aspirate reagents from the plate before dispensing the wash solution.
- Do not allow the plate to dry between plate washings and prior to the addition of reagents.
- Do not scrape the surface of the plate as this will remove the antigen/antibody bound to the surface and cause inconsistent or inaccurate results.
- After the final wash, the plate should be inverted and tapped on absorbent paper to remove the last traces of Wash Buffer. If needed, the volume of wash can be increased (i.e 350 or 400 µl) per well and a soaking time (i.e:1 minute) can be added between washes. After tapping out plates, check paper towels for any evidence of colour. This

may indicate that the plates were not washed properly and there are reagents remaining in or around the wells.

- When testing milk, albumin, whole blood or yolk samples, take extra care to inspect the wells. Because of their protein or fat composition, these sample types are sometimes more difficult to wash from the wells and may require the maximum recommended number of wash cycles. Be careful that there is no fatty ring left in the well after washing. To avoid fat residues, it is possible to include a soaking time of 2 – 5 minutes between washes.

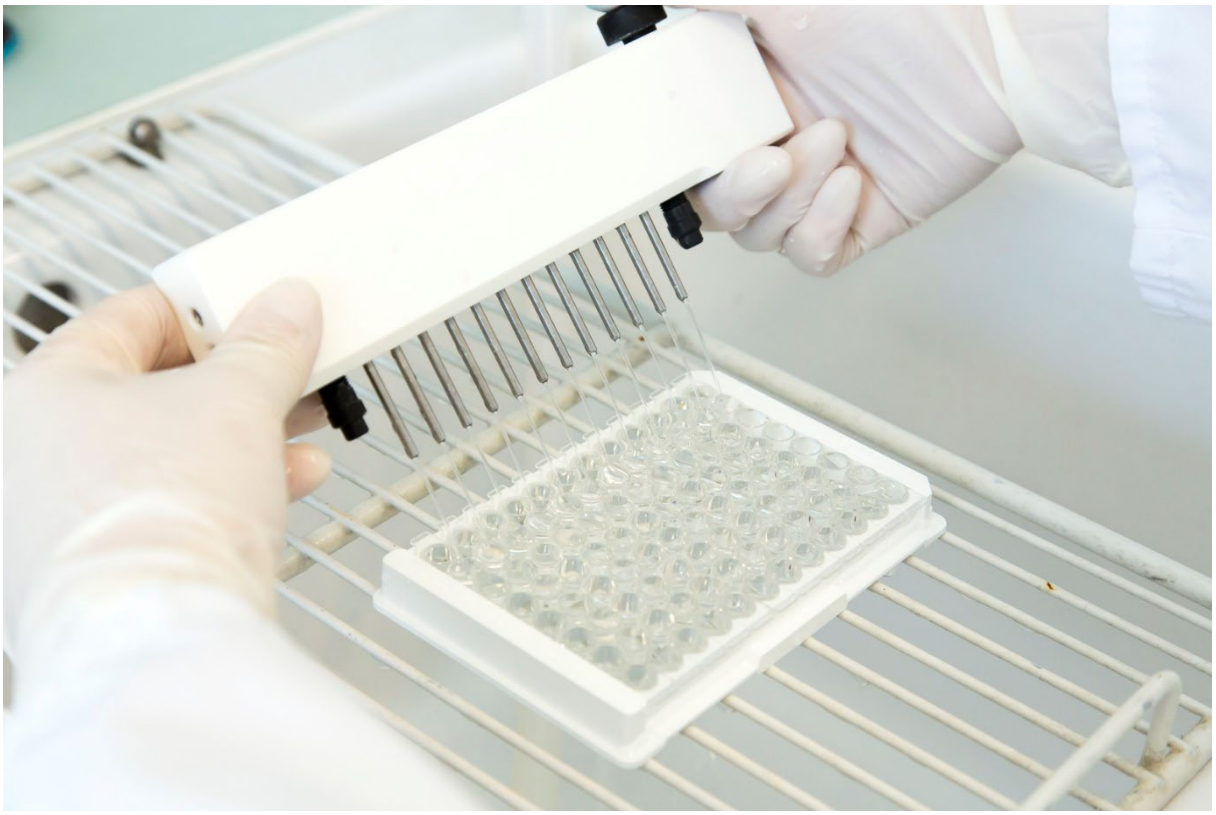
Manual Washing

- Manual methods include washing by multichannel pipettes or wash bottles (not recommended). These methods do not allow for efficient washing of plates and may cause high or inconsistent background.
- Ensure complete filling of microwells.
- Aspirate or shake out the contents of the microwells. If shaking out the plates, turn the plates completely over and empty twice with two swift movements before proceeding to the next wash. Ensure that wells are empty between wash cycles.
- Work quickly so the time from washing the first well/row to the last is minimal. If the time is too long, the empty wells may dry out and the last wells will have a longer incubation than the first wells.
- Avoid drying of the wells between washes or before addition of the next reagent.
- Tap the plate on paper towel before adding conjugate or substrate. Check the paper towels for any evidence of colour. This may indicate that the plates were not washed properly and there are reagents remaining in or around the wells.

Semi-Manual Washing

- At IDvet, the semi-manual wash system is a glass erlenmeyer flask with a comb dispense system:

- Ensure complete filling of microwells.
- Turn the plates completely over and empty twice with two swift movements before proceeding to the next wash. Ensure that wells are empty between wash cycles.
- Avoid drying of the wells between washes or before addition of the next reagent.
- Tap the plate on paper towel before adding conjugate or substrate. Check the paper towels for any evidence of colour. This may indicate that the plates were not washed properly and there are reagents remaining in or around the wells.



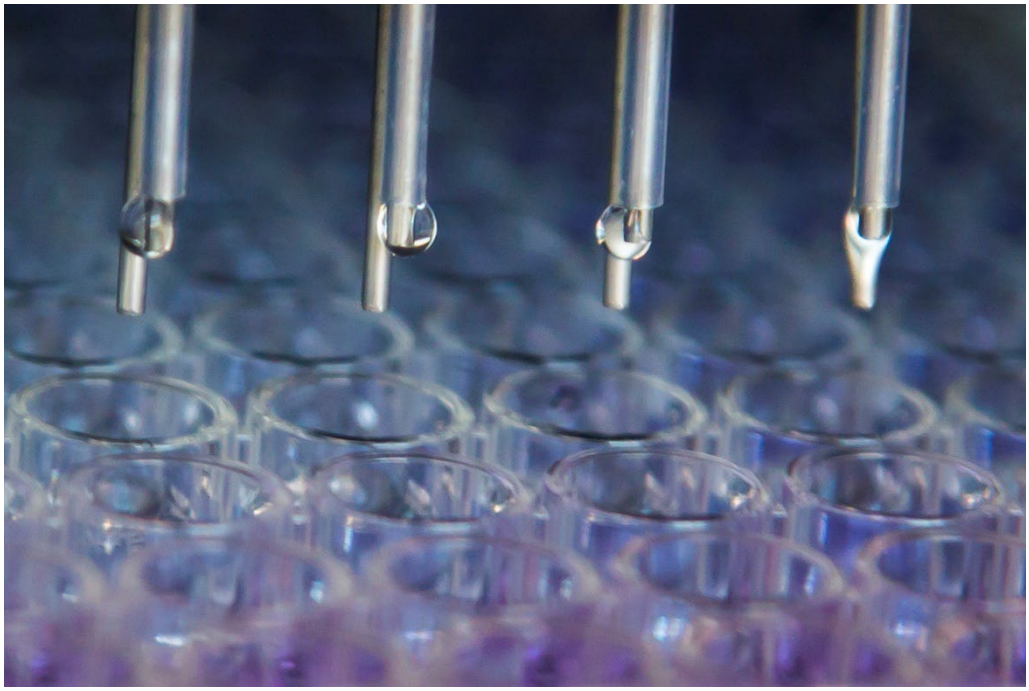
Automated or Semi-automated Wash Systems

- In general, an automated or semi-automated wash system in proper working order will provide more consistent washing than manual methods.
- Check that all the dispensing needles are dispensing with a smooth, steady stream and that all aspiration ports aspirate uniformly.
- The plate-washing technique should be consistent from plate to plate and from row to row within a plate.
- Make sure your wash system is properly cleaned and maintained. Microbial build up in tubes, for example, can negatively affect results.
- Automated washers should be programmed to complete at least 3 wash cycles.
- Washers must be correctly calibrated to ensure complete filling and emptying of microwells between each wash.
- The height of plates may vary between manufacturers. Thus, aspiration parameters must be adapted to the trademark of plates included in the kit (see following chapter).

- **If you use a plate washer, remember to check the following key parameters:**
    - **mode**: you should use the plate mode (add wash to all strips, then aspirate all strips). The strip mode should not be used, in which wash is added to a strip, and then aspirated immediately.
    - **aspiration**: use crosswise aspiration if possible (double aspiration in each corner of the well), in order to ensure removal of all wash;
    - ensure that the amount of **residual liquid** between washes and after washing is as low as possible (test by tapping the plate against paper towel after each aspiration);
    - **aspiration height:** to obtain this parameter, please refer to the specifications provided by the plate manufacturer (Greiner, Nunc etc.). For Greiner strip plates used by IDvet, the aspiration height should be 3.2 mm. Check visually that the wash needles are as close to the bottom of the wells as possible, without touching. Contact info@id-vet.com if you require similar information for our whole plate format (Nunc brand).

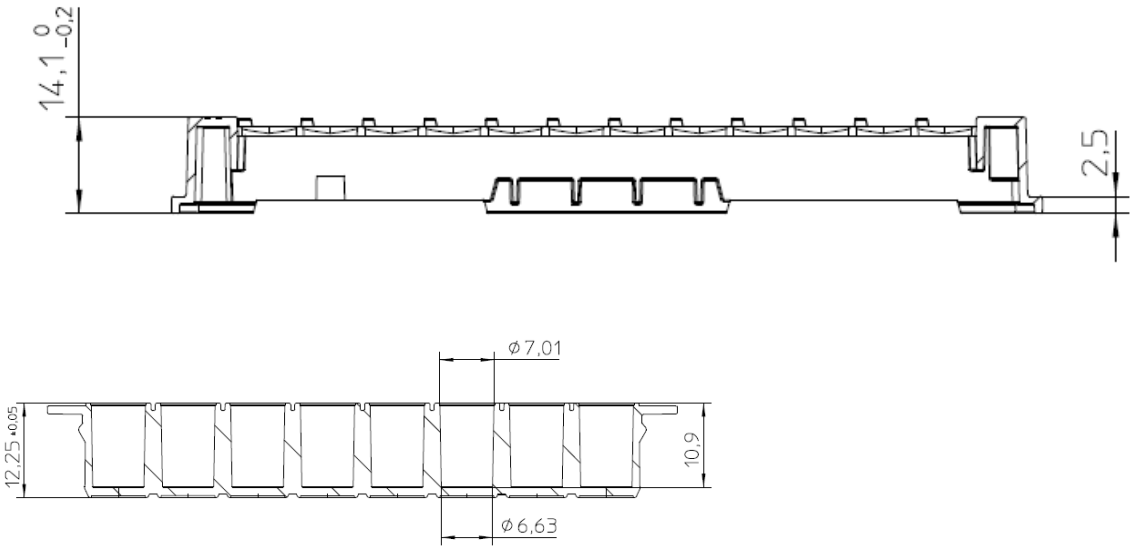
- In order to verify the effectiveness of the plate washer, we recommend that from time to time, you **run a complete wash cycle using wash solution containing blue food colouring**. If the plate washer is not performing correctly, you will see blue traces on the plate after the wash cycle, indicating the need for maintenance. Stop the washer after the aspiration step. Pipette the residual volume, which should be less than 5 µl. Refer to the washer's instructions for use for more information.

- Perform **maintenance and decontamination procedures** at regular intervals. Contact the plate washer's technical support team for more information regarding the implementation of these preventive measures.



For the Greiner strip plates supplied by IDvet, Greiner provides the following information for washer settings:

Page 1 of 2	CUSTOMER DRAWING	greiner bio-one
Revision 3	PS Strip Plate, 96 Well, F - Bottom	



Height of bottom of wells = Plate height – strip height = 14.1-10.9 = 3.2 mm.

Height of bottom of wells = 3.2 mm

Will the sterility or the quality of the water used to reconstitute the wash solution affect test results?

- While we recommend that you use distilled / deionised the water to reduce bacterial load, it is not necessary to use sterile water or to store distilled / deionised water in a sterile environment.

- In these conditions, the 1X wash solution will not be sterile, but its low level of bacteria will not affect results.

- A high level of bacteria in the water, however, caused by the progressive contamination of the wash solution or the use of dirty glassware, can lower the quality of results.

- In this case, you will notice a reduction in the difference between the positive and negative signals, and an increase in the background signal.

- Generally, it is recommended that the 1X wash solution be prepared at the beginning of the week, and not be conserved for more than 5 days (ex. from Monday to Friday).

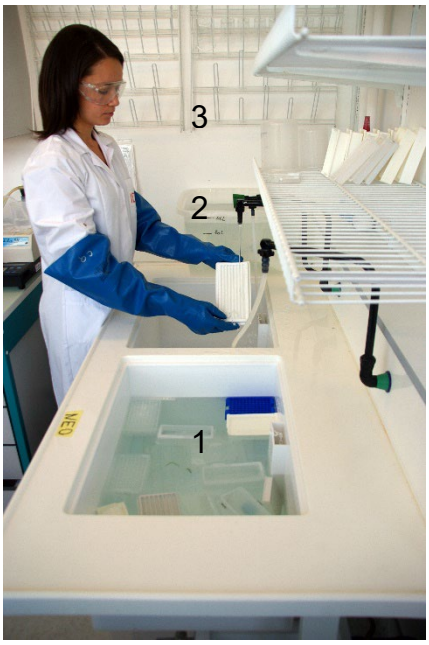
- It is essential that the glassware and the wash system (manual, semi-automatic or automatic) be very clean. The wash system should be thoroughly cleaned at least once a week - otherwise, it is pointless to prepare fresh 1X wash solution!

Incorrect cleaning of vessels can impact results!

It is important to correctly clean all dishware, and in particular the dishware used for reagent distribution, in order to obtain correct results.

We also recommend reserving a reservoir specifically for the substrate in order to avoid problems with "blue" substrate.

Recommended washing procedure:



- Dishware and buffer reservoirs should be put in contact with an alkaline cleaning agent (we use **Neodisher FT diluted 1:1000 in distilled water**) for at least one hour (1). Be sure to remove any labels and markings with an abrasive sponge to avoid inactivating the Neodisher.
- Rinse three times with hot or cold tap water (2).
- Immerse the vessel in deionised water and soak for at least 1 hour (3).
- **Note:**
    - Problems can arise if dishware is just simply rinsed with water, and the aforementioned procedure is not respected.
    - This cleaning protocol is particularly important if the vessel was used for the conjugate.
    - The Neodisher solution should be changed weekly.
    - Wear appropriate protective wear (lab coat, glasses, gloves..)

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