Chapter 2

Microplate Washer

GMDN Code	**17489**
ECRI Code	**17-489**
Denomination	**Micro-plate washer**

The microplate washer or "plate or ELISA washer" is designed to perform washing operations required in the ELISA technique. The microplate washer performs the washing of the ELISA plate's wells during the different stages of the technique.

PHOTOGRAPH OF MICROPLATE WASHER

Photo courtesy of BioRad Laboratories

PURPOSE OF THE MICROPLATE WASHER

The microplate washer has been designed to supply cleaning buffers required for the ELISA technique in a controlled manner. In the same fashion, the equipment removes from each well, substances in excess from the reaction. Depending on the test performed, the washer can intervene from one to four times, supplying the washing buffer, agitating and removing the unbound reagents[1] until the programmed times and cycles are completed. The washer has of two reservoirs; one for the washing buffer, the other for the waste generated during the washing process.

OPERATION PRINCIPLES

The microplate washer has been designed to perform washing operations in the ELISA technique. The equipment possesses at least, the following subsystems which vary depending on the manufacturer's design.

- ***Control subsystem.*** Generally, the washer is controlled by microprocessors allowing programming and controlling steps to be performed by the washer such as: number of washing cycles[2] (1–5); expected times; supplying and extracting pressures; plate format (96–384 wells); suction function adjustment according to the type of well[3] (flat bottom, V bottom or rounded bottom or strips used); volumes distributed or aspirated; the soaking and agitation cycles, etc.
- ***Supply subsystem.*** In general, this comprises a reservoir for the washing solution; one or several pumps; usually a positive displacement type syringe and a dispenser head that supplies the washing solution to the different wells by means of needles. The head usually comes with eight pairs of needles for washing and aspirating simultaneously the wells of the same row (the supply and extraction sub-systems converge on the head). There are models with twelve pairs of needles and others that conduct the washing process simultaneously in all the wells. Some washers offer the possibility of working with different types of washing solutions, performing the solution changes according to the program entered by the operator.

[1] See a brief explication of the ELISA technique in Chapter 1, *Microplate Reader*.

[2] The exact number of washing operations required depends on the assay used. This is explained in each manufacturer's test instruction manual.

[3] If the bottom is flat, the suction needle is located very close to one of well's faces; if it is rounded or V-shaped, the suction needle is centered.

- ***Extraction or suction system.*** This requires a vacuum mechanism and a storage system for gathering the fluids and waste removed from the wells. The vacuum may be supplied by external and internal pumps. Extraction is done by a set of needles mounted on the washer/dryer's head. The number of needles varies from one to three, according to the washer model used.

  If it uses only one needle, the washing and extraction operation is done with this single needle. If it uses two needles, one is used for supplying the washing solution and the other for extraction. If it uses three needles, the first is used for supplying the washing solution, the second for extraction and the third for controlling (extracting) any excess volume in the well. Generally, the extraction needle is longer than the supply needle, which enables it to advance (vertically) up to a height ranging between 0.3 and 0.5 mm from the bottom of the well.
- ***Advance sub-system.*** This is composed of a mechanism which moves the supply and extraction head horizontally to reach each well in the ELISA plate. When the horizontal movement to the following row occurs, there is a vertical movement towards the well to dispense or extract the washing solution. There are washers which carry out these operations in a simultaneous manner.

The sub-systems previously described are shown in Figure 2. Figure 3 shows the different types of wells most commonly found in microplates. Each kind of well is suitable for a particular type of test.

Washing process

The washing of the microplate is one of the stages of the ELISA technique. Special solutions are used in the washing steps. Among those most commonly used is phosphate buffer solution or PBS. The phosphate buffer solution has a stability of 2 months if kept at 4 °C. It is estimated that 1 to 3 litres of solution is required for washing one microplate and that 300 *µl* is used in each well per cycle. Washing can be done manually, but it is preferable to use an automated microplate washer for a better throughput and to minimize handling of potentially contaminated substances.

Among the washing processes used by microplate washers are featured:

- ***Aspiration from top to bottom.*** When the aspiration phase is initiated, the needles move vertically and the aspiration is initiated immediately as these enter into the liquid. The process continues until the needles reach their lowest position very close to the bottom of the wells. At this point they are stopped in order to avoid suctioning the air that should flow against the interior lateral walls of the wells. This type of aspiration prevents air currents from drying the bound protein on the surface of the wells.

Figure 2. Microplate washer

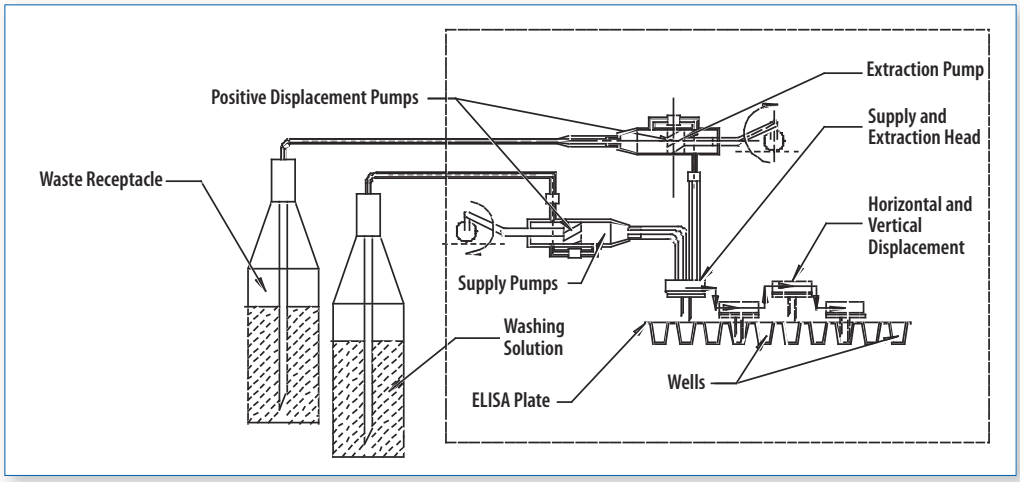
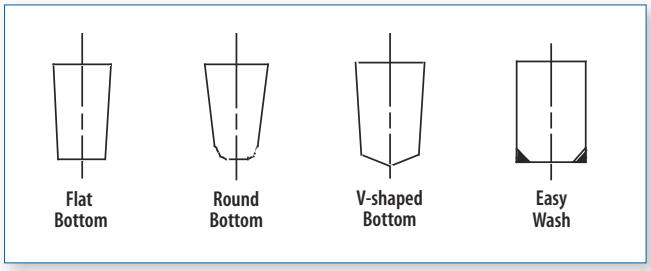


Figure 3. Well profiles



- ***Simultaneous distribution and aspiration.*** In certain types of washer, the washing and aspiration systems operate simultaneously, generating a controlled turbulence inside the well which removes the unbound substances during the incubations.
- ***Aspiration from the base of the wells.*** In this system, the aspiration of the fluid contained in the wells is performed initially with the aspiration needles in a position very close to the bottom, immediately beginning a suctioning cycle, usually time-controlled. This system may aspirate air if there are differences in the levels of the tanks.

Washer calibration

The microplate washer is critical for guaranteeing that the ELISA technique performs as expected. The alignment to be taken into account for the effective functioning of the equipment is presented next:

- ***Position of the needles (supply and aspiration head).*** The horizontal and vertical position adjustment with respect to the wells must be verified carefully. If the plate has flat bottom wells, the supply needle must be checked to see that it is situated very close to the well's wall. If the bottom is round or V-shaped, the suction needle should be located in the centre of the well: upon the vertical movement, a needle-base distance is maintained in the well, usually between 0.3 to 0.5 mm. The needles must never be allowed to touch the bottom of the wells to avoid mechanical interferences between the needle point and the well's base during the aspiration function.
- ***Aspiration time.*** Appropriately adjust the aspiration time so that a solution film adhered to the well's wall can flow towards the bottom. Avoid very long time lapses to prevent the coating on the wells from drying up. Check that the suction system's needles are clean (free of obstructions).
- ***Distributed Volume.*** Check that the volume distributed is as close as possible to the maximum capacity of the well; confirm that all the wells are filled uniformly (at the same level). Verify that the distributing needles are clean (free of obstructions).
- ***Vacuum.*** The suctioning system must be calibrated efficiently. If the vacuum is too strong, the test can be altered. In fact, it could dry out the wells and considerably weaken the enzyme activity in the wells and completely alter the test result. The majority of washers function with a vacuum ranging between 60 and 70% of atmospheric pressure. In some washers, the vacuum is made in an external pump which operates as an accessory of the washer. Its operation is controlled by the washer, which means that the vacuum pump operates only when required.

Washing process verification

To verify that the washing process is done according to the specifications of ELISA techniques, manufacturers of ELISA tests have developed procedures to be carried out regularly. One of the controls[1] is based on using the peroxidase reagent, which is dispensed using a pipette in the plate wells to be read at 405, 450 and 492 nm. At once the wells are washed and a colourless substrate is added (TMB/H_2O_2–Tetramethylbenzidine/Hydrogen Peroxide). Whatever conjugate remains will hydrolyze the enzyme and the chromogen will change to blue. After stopping the reaction with acid, the TMB will turn yellow again. The resulting colour intensity is directly related to the washing process efficiency.

INSTALLATION REQUIREMENTS

For the microplate washer to operate correctly, the following is necessary:

1. A clean, dust-free environment.
2. A stable work table located away from equipment that generates vibrations, (centrifuges, and agitators). It must be of a suitable size to locate the necessary complementary equipment: reader, incubator, distributor and computer with its peripheral attachments at the side of the microplate washer.
3. An electric outlet in good condition with a ground pole and, an electrical connection which complies with the country's or the laboratory's norms and standards. In the countries of the Americas, the 110 V and 60 Hz frequency is generally used. In other parts of the World, the 220-240 V and 50/60 Hz frequency is generally used.

ROUTINE MAINTENANCE

The routine maintenance described next focuses exclusively on the microplate washer. Maintenance of the microplate reader is dealt with in the Chapter 1.

Basic maintenance

Frequency: Daily

1. Verify the volume distributed.
2. Test the filling uniformity.
3. Verify the aspiration sub-system's efficiency.
4. Confirm the cleaning of the supply and extraction needles.
5. Clean the washer with distilled water after use, to remove every vestige of salt in the supply and extraction sub-systems' channels. The needles may be kept submerged in distilled water.
6. Verify that the body of the washer has been cleaned. If necessary, clean the exterior surfaces with a piece of cloth, moistened with a mild detergent.

[1] Procedure developed by PANBIO, *ELISA Check Plus*, Cat. Nº E-ECP01T.

Preventive maintenance

Frequency: Quarterly

1. Disassemble and clean the channels and connectors. Verify their integrity. If leaks or any vestiges of corrosion are detected, adjust and/or replace.
2. Verify the integrity of the mechanical components. Lubricate according to the manufacturer's instructions.
3. Test the adjustment of each one of the sub-systems. Calibrate according to the manufacturer's recommendations.
4. Confirm the integrity of the electrical connector and the inter-connection cable.
5. Clean the washer with distilled water after using it in order to remove every vestige of salt in the supply and extraction subsystems' channels.
6. Verify the integrity of the fuse, and that its contact points are clean.

Note: Trained technical personnel must carry out maintenance of the control system. If necessary, call the manufacturer or representative.

TROUBLESHOOTING TABLE

PROBLEM	PROBABLE CAUSE	SOLUTION
Upon completion of washing, residual solution remains in the wells.	The washer extraction system demonstrates failure.	Verify if the vacuum system is functioning at the appropriate pressure.
	The conducts/pipes of the vacuum system are of a different diameter than that recommended.	Check that the diameter of the channels corresponds to the recommendation by the manufacturer.
	The suction line shows obstructions.	Verify that the vacuum lines are clean.
	The container for storing the waste is full.	Confirm the waste recipient's level.
	The line filter is damp or blocked.	Verify the state and integrity of the suctioning system's filter.
	The needles' points are not placed correctly and do not reach the bottom of the wells.	Examine the placement of the needles' points.
	A different microplate is used in the test.	Verify the type of plate required for the test.
	The washer has not been purged sufficiently.	Check the purging process.
	The operator has not followed the manufacturer's instructions correctly.	Examine the process recommended by the manufacturer. Carry out the required adjustments.
	The plate placed in the washer is incorrectly aligned.	Check the placement of the plate in the washer.
The washing cycle is performing inadequately.	The washing solution reserve is exhausted.	Examine the cleaning solution storage receptacle. Replace the volume missing.
	The washer was not purged sufficiently at the beginning of the work cycle.	Clean adequately in order to homogenize the humidity in each one of its components and to eliminate air bubbles.
	The volume of washing solution distributed has been programmed erroneously.	Verify the required volume for each type of test and for each plate.
	The plate was placed incorrectly in the washer.	Check the correct installation of the plate in the washer.
	The cycle setting was incorrectly selected.	Review the cycle setting recommended for each type of plate.
	The plates used are different from those recommended by the manufacturer.	Verify that the plates used are completely compatible with the washer.
	The fluid level in the wells is inadequate.	
	The washing solution supply tube is not of the diameter or thickness specified by the manufacturer.	Check the manufacturer's specifications. If necessary, correct.
	The pressure is insufficient for delivering the adequate amount of washing solution.	Check the supply system and supply channels, there might be an obstruction in the filling line.
The washing container shows fungal and bacterial growths.	The system is not used frequently.	Check the procedures used for preventing fungal and bacterial growth.
	An adequate control procedure (disinfection) is not used.	Check the procedures used for preventing fungal and bacterial growth.
	The tubes and connectors are not changed with the required frequency.	Verify the change frequency suggested by the manufacturer and or the technical department.
	The washing solution has been contaminated.	Confirm the procedures used in the preparation and management of the washing solution with the aim of determining the cause of contamination and eliminate it.
	Maintenance has not been carried out according to its schedule.	Check the dates planned for carrying out maintenance. Inform those responsible.

BASIC DEFINITIONS

Buffer. A solution containing either a weak acid and its salt or, a weak base and its salt, which makes it resistant to changes in pH at a given temperature.

PBS. One of the solutions used to perform washing operations in ELISA tests. PBS is the acronym for Phosphate Buffer Solution. This is made of the following substances: *NaCl, KCl, $NaHPO_4 2H_2O$ and KH_2SO_4*. The manufacturers supply technical bulletins which indicate the proportions and instructions for preparing PBS. In general, one part of concentrated PBS is mixed with 19 parts of deionised water.

Plate (ELISA). Consumable with standard dimensions, designed to hold samples and reactions for the ELISA technique. In general, these have 96, 384 or 1536 wells and are made of plastics such as polystyrene and polypropylene. There are plates specially treated to facilitate the performance of the tests.

Positive displacement pump. A pump adjusted by a plunger moving along a cylinder. The mechanism is similar to that of a syringe. It is equipped with a set of valves for controlling the flow to and from the pump.

TMB/H_2O_2. (Tetramethylbenzidine/hydrogen peroxide). A set of reagents used for verifying the quality of washing done on the wells used in the ELISA technique.