

How to dispose reagent solutions, well-plate, etc., after assay

In general, dispose them according to the rules enacted by the state, city or institution.

Else, in the absence of such authentic rule, dispose them following the procedure indicated below.

[Washing buffer]

The constituents of the washing buffer are detergent and phosphate buffer. Discard it with plenty tap water. Discard the rest of the concentrated washing buffer remained unused in the same way.

[Solutions of standard preparation, HRP-antibody conjugate, biotin-labeled antibody, and HRP-avidin conjugates]

Discard them with plenty tap water. Dispose the rest of the buffer remained unused in the same way.

[Chromogenic substrate (TMB) solution]

Hand over the rest of the solution unused to a waste handler as an organic solvent.

[Reaction stopper: 1M H₂SO₄]

Hand over the rest of the solution unused to a waste handler as acidic solution.

Else, neutralize sulfuric acid by adding those neutralizing chemicals as shown below, then discard with enough tap water into a sink. Before neutralization: First, transfer the reaction stopper solution to a transparent glass conical flask or conical beaker of enough volume (10 times of residual reaction stopper).

Wash the bottle used as a container of reaction stopper with plenty of water, and discard it as a plastic waste.

During the neutralization procedure, never forget to wear face and eye protective glasses or goggles. If received splash of the reaction mixture, wash face and eye with plenty tap water as soon as possible and consult a doctor.

Please, be careful for heat during neutralization process!

Procedure of neutralization and caution

Prepare 0.5M sodium hydroxide (NaOH) solution, and 0.5M sodium bicarbonate (NaHCO₃) solution. Estimate the volume of the residual reaction stopper.

Procedure: If the volume of residual reaction stopper is V ml, first add 3.5 x V ml of NaOH solution little by little, then add V ml of NaHCO₃ solution little by little.

Caution: Be careful for the heat production during neutralizing reaction.

Also, the bubbles of carbon dioxide will emerge when NaHCO₃ solution is added. Do not add large amount of neutralizing solutions at once. Do not reverse the order of addition.

In the case of sodium bicarbonate, pH of the mixture will stay below pH 10 even if excessive amount is added. So, the neutralized solution can be discarded with plenty tap water into a sink.

You can neutralize sulfuric acid solution by sodium bicarbonate solution alone, however, it gives considerable amount of carbon dioxide (if 10ml of 1M sulfuric acid is neutralized with sodium bicarbonate, 450ml (880mg) of carbon dioxide will be formed. To minimize carbon dioxide, the method with combined use of sodium hydroxide and sodium bicarbonate is proposed.

[Treatment of well-plate and solution in the well after assay]

Aspirate solution in the well by using a bottle with jet nozzle (prepared by cutting the tubing within the bottle) or a tip connected to an aspirator via a trap bottle.

Hand over the solution recovered to a waste handler as acidic solution, else neutralize by adding the neutralizing agents as similar ways stated in the section of reaction stopper, then discard with plenty tap water into a sink. The sulfuric acid concentration of the solution in the well is one half of the reaction stopper, i.e. 0.5M. So, add $1.8 \times V$ ml of NaOH solution, then $0.5 \times V$ of NaHCO_3 solution.

(V: Volume of recovered solution from wells)

Hand over the well-plate aspirated off the contents, after washing with plenty tap water, to a medical waste handler, or else, dispose as a plastic waste after washing and sterilization by autoclave or by dipping in the aqueous solution (>1%) of sodium hypochlorite for more than 1 hour, then dispose as a plastic waste.

[Sample containers and tips for sample treatment]

Hand over sample containers and pipette tips used for sampling to a medical waste handler, or else sterilize them by dipping in 1% formalin, 2% glutalaldehyde, or more than 1% of sodium hypochlorite for more than 1 hour, and dispose as plastic wastes.

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