10% Neutral Buffered Formalin

Contents

[Purpose 1](#_Toc508092426)

[Scope 1](#_Toc508092427)

[Responsibilities 1](#_Toc508092428)

[Supervisors 1](#_Toc508092429)

[Workers 2](#_Toc508092430)

[Equipment Needed 2](#_Toc508092431)

[Preparing Vytac Waste Bottle 2](#_Toc508092432)

[Fixing Cells in a Dish 2](#_Toc508092433)

[Re-suspending Cells in Formalin 3](#_Toc508092434)

[Fixing Tissues in Formalin 3](#_Toc508092435)

[Post-Fixation Dissection of Tissues Fixed in Formalin for FFPE 3](#_Toc508092436)

[Training 4](#_Toc508092437)

# Purpose

<<<< This SOP is left in DOCX format so that you may edit it for your own laboratory and your own procedures using 10% neutral buffered formalin>>>

The purpose of this SOP is to lay out the responsibilities, equipment and procedures required for the use of 10% neutral buffered formalin.

# Scope

This SOP applies to all persons prescribing and requiring to use 10% neutral buffered formalin.

# Responsibilities

## Supervisors

Supervisors are responsible for:

* **Reviewing this SOP on a regular basis. Review is to consider and mitigate the risks of spill, loss of containment and exposure or other harm. Refer to Performing Risk Assessments SOP.**
* Ensuring the equipment is in good working order.
* Ensuring all persons are trained on the Safety Data Sheet and are aware of the risks related to the use of 10% neutral buffered formalin.
* Ensuring that all workers under their supervision are trained on and are proficient in performing the steps of this SOP.

## Workers

Workers are responsible for:

* Following this SOP as approved by their supervisor
* Reporting any broken equipment or spills immediately to their supervisor.

# Equipment Needed

* Vytac® <https://www.thermofisher.com/order/catalog/product/5401TS>
* Rinsed, plastic media bottle for use as a waste bottle
* Funnel
* Filter paper or blotting paper

# Preparing Vytac Waste Bottle

1. Vytac requires a 1/5 dilution of Vytac:Formalin
2. For a 500ml bottle, use a black sharpie to mark 400ml and 500ml.
3. Label 400ml line as FORMALIN
4. Label 500ml line as VYTAC.
5. Collect waste formalin in a fume hood until it reaches 400 ml.
6. Fill the bottle to the 500ml mark with Vytac.
7. Close bottle and mix.
8. Allow to stand per Vytac instructions.
9. Ensure disposed liquid is pre-filtered using a funnel and filter or blotting paper.
10. Dispose neutralized, filtered solution down sink per Vytac instructions.
11. Filter should be left to dry in fume hood and then discarded in biohazard waste due to visible tissue bits and residue.

# Fixing Cells in a Dish

1. Calculate the amount of 10% neutral buffered formalin required.
2. In a fume hood, decant that amount into a screw cap 50ml falcon tube.
3. Small amounts of chemicals are allowable in a BSC. The key is to keep the containers closed all times.
4. Bring falcon tubes to BSC and spray-in per lab SOPs.
5. Aspirate and wash cells per lab SOPs.
6. Loosen the lid of 50ml tube containing formalin.
7. Apply required amount of formalin to each well or dish. Label dishes with ‘10% NBF’.
8. Re-cap formalin tube.
9. Bring dishes to fume hood.
10. Carry out rest of procedure in a fume hood.
11. Decant extra or waste formalin in Vytac waste bottle.

# Re-suspending Cells in Formalin

1. Calculate the amount of formalin required.
2. In a fume hood, decant the required amount into 50ml falcon tubes.
3. Bring falcon tubes to BSC.
4. Prepare cell suspension per lab experimental protocols.
5. Spin and resuspend cells in formalin per lab experimental protocols.
6. Fix cells per lab experimental protocols.
7. Spin and resuspend cells in other buffer per lab experimental protocols.
8. Collect extra and waste formalin in Vytac waste bottle.

# Fixing Tissues in Formalin

1. Calculate the minimal amount of formalin required to fix each sample.
2. In a fume hood, decant the required amount into appropriate sized, leak-proof tubes.
3. Bring tubes to tissue-harvesting location.
4. Before each harvest, open the appropriate tube, but keep cap on loosely.
5. Place each sample in a separate tube, pre-rinsing tissue with PBS if necessary.
6. Close tube tightly.
7. Fix tissues in formalin according to lab experimental protocols.
8. Ensure each sample contains the notation ‘10% NBF’ on tube.

# Post-Fixation Dissection of Tissues Fixed in Formalin for FFPE

1. Perform if allowable for downstream applications.
2. Bring tissue samples to fume hood.
3. Decant formalin from each tissue sample into Vytac waste bottle.
4. Wash each tissue sample with sterile PBS overnight.
5. Decant PBS wash into Vytac waste bottle.
6. Repeat wash and decant if necessary.
7. Dissect tissues in fume hood into pre-labelled cassettes per experimental protocols.
8. Collect waste tissue bits into a plastic bag. Dispose in biohazard waste.
9. Place cassettes into 70% ethanol per experimental protocols.
10. Submit for histology per department procedures.

# Training

1. Each lab member is to read, agree and sign off on this SOP.
2. Upon revision or re-write, this SOP is to be re-circulated and re-signed by all lab members.