

Reagent Preparation [Ziehl Neelsen Staining-1]

Name of reagent: 1% Carbol fuchsin	Lot number:	Date of preparation:	Total Amount prepared:	Expiration Date:	Prepared by:
Ingredients	Supplier/catalog number	Lot number	Amount added	Technician/Technologist	Date
Basic fuchsin			1 g		
Absolute Ethanol			10 ml		
Phenol crystal			5 g		
Distilled water up to			100 ml		

Instructions for preparation of 1% Carbol fuchsin:

1. Using a digital balance weigh out 1 g of Basic fuchsin in a sterile 100 ml flask.
2. Add 100 ml of absolute alcohol and dissolve the dye by placing it in a water bath at 60° C. Avoid direct heating (Solution 1).
3. Using a digital balance weigh 5 gm of phenol crystals in a balance and transfer it to 100 ml Erlenmeyer flask
4. Add 50 ml of distilled water and dissolve the phenol crystal. Gentle heat may be required. (solution 2)
5. Mix solution 1 and solution 2 and transfer the contents into a 100 ml measuring cylinder
6. Add distilled water to make up the final volume to 100 ml.
7. Pour the solution through filter paper (whatman No 1) and store filtered solution in an amber colored bottle.
8. Label bottle with name of reagent (1% Carbol Fuchsin), lot number, date of preparation, expiration date & storage temperature.
9. Tighten caps & store at room temperature.

[Note: Store the reagent upto 6 months from preparation date. Occasionally filter Carbol fuchsin if precipitate forms.]

Reagent Preparation [Ziehl Neelsen Staining-2]

Name of reagent: 25% Sulphuric acid	Lot number:	Date of preparation:	Total Amount prepared:	Expiration Date:	Prepared by:
Ingredients	Supplier/catalog number	Lot number	Amount added	Technician/Technologist	Date
Conc. H ₂ SO ₄			25ml		
Distilled water			75 ml		

Instructions for preparation of 25% Sulphuric acid:

1. Using a 100 ml measuring cylinder measure out 75 ml of distilled water in 500 ml conical flask and submerged the flask in a bowl of cold water.
2. Using a 50 ml measuring cylinder measure out 25 ml of conc. Sulphuric acid and carefully add concentrated Sulphuric acid to the water. This mixture will heat up, so always place the flask in a bowl of cold water while diluting the acid.
3. Mix gently and store it in amber colored bottle glass bottles with caps.
4. Label bottle with name of reagent (25 % Sulphuric acid), lot number, date of preparation, expiration date & storage temperature.
5. Tighten caps & store at room temperature. Acid prepared can be stored upto 12 months from the date of preparation.

[Note: Always add acid to water. Never add water to acid.]

Reagent Preparation [Ziehl Neelsen Staining-3]

Name of reagent: 0.1% Methylene blue	Lot number:	Date of preparation:	Total Amount prepared:	Expiration Date:	Prepared by:
Ingredients	Supplier/catalog number	Lot number	Amount added	Technician/Technologist	Date
Methylene blue			0.1 g		
Distilled water			100 ml		

Instructions for preparation of 0.1% Methylene blue:

1. Using a digital balance weigh out 0.1 g of methylene blue in a sterile 500 ml flask and dissolve it in 100 ml of distilled water.
2. Use a stirring bar or swirl solutions to mix.
3. Pour the solution through filter paper (whatman No.1) and store filtered solution in an amber colored glass bottles with caps.
4. Label bottle with name of reagent(0.1 % Methylene blue), lot number, date of preparation, expiration date & storage temperature.
5. Tighten caps & store at room temperature.

[Note: Store the reagent upto 6 months from preparation date.]

Quality Control

Stain a positive control and a negative control slide using the new batch of staining reagent and observe for reagent quality. Smear quality good= pass, Smear quality poor= Fail.

Performed by:	Date stained:	Staining quality [Good/poor]			Final results (Smear quality Good or poor)	Pass/Fail & Date
		Color of AFB stained	Background	Counter staining		
Positive Control						
Negative Control						