



Biochemical Diagnostics, Inc.

REAGENT PREPARATION

Please take proper health and safety precautions when working with laboratory chemicals. Become familiar with any potentially hazardous or toxic characteristics of chemicals before using them in your reagent preparations.

BUFFERS

ACETATE BUFFER, pH 4.0

Prepare a 0.2M Sodium Acetate Solution. Weigh 27.2 gm of Sodium Acetate Trihydrate into a one liter volumetric flask. Add 800 mL of Deionized Water. Mix and dissolve. Bring the pH down to 4.0 with Glacial Acetic Acid. Finally adjust the volume to one liter with Deionized Water.

ATP Releasing Reagent

ACETATE BUFFER, pH 5.0

Prepare a 0.2M Sodium Acetate Solution. Weigh 27.2 gm of Sodium Acetate Trihydrate into a one liter volumetric flask. Add 800 mL of Deionized Water. Mix and dissolve. Bring the pH down to 5.0 with Glacial Acetic Acid. Finally adjust the volume to one liter with 0.1. water.

HEPES, .025M Weigh 6.0 gm of Hepes (N-Hydroxyethyl PIPERAZINE N2 ETHANESULFONIC ACID). Dissolve in Deionized Water and bring it up to one liter. Adjust the pH to 8.2 with 2N Sodium Hydroxide.

PHOSPHATE BUFFER, pH 6.0

Prepare 0.25M Potassium Phosphate, Monobasic, (34 gm KH_2PO_4 /liter water). Initial pH is approximately 4.8. Adjust the pH upward to 6.0 with 0.25M Sodium Phosphate, Dibasic, (35.5 gm Na_2HPO_4 /liter of water).

PHOSPHATE BUFFER, pH 8.4

Prepare 0.25M solution of Sodium Phosphate, Dibasic, in Deionized Water. (Initial pH approx. 9.25) Bring the pH down to 8.4 with the slow addition of a 0.25M solution of Potassium Phosphate, Monobasic, in water.

PHOSPHATE BUFFER, pH 9.1

Prepare a 0.25M solution of Sodium Phosphate Dibasic in Deionized Water . Adjust the pH with Potassium Phosphate Monobasic (if the pH is low use 2N Sodium Hydroxide to bring it up to 9.1).

PHOSPHATE BUFFER, pH 10.0

Prepare as Reagent F and increase the pH with 2N Sodium Hydroxide to pH 10.0.

PHOSPHATE BUFFER, pH 6.0 with Column Activator

Measure 70 mL of n-Propanol and bring it up to one liter with pH 6.0 Phosphate Buffer (Reagent D)

PHOSPHATE BUFFER, pH 9.1 with Column Activator

Use only Potassium Phosphate Dibasic, (K_2HPO_4) because the Sodium Salt will precipitate in n-Propanol. Weigh 43.5 gm of K_2HPO_4 . Mix and dissolve in approx. 900 mL of Deionized Water. Add 70 mL of n-Propanol and adjust the pH to 9.1 with KH_2PO_4 (see Reagent D). Bring the volume to one liter with water.

7N POTASSIUM HYDROXIDE

Slowly add 450 gm of Potassium Hydroxide pellets to a volumetric flask containing 500 mL of Deionized Water. This is done in a cool water bath. When all the pellets are dissolved and the temperature has come down to room temperature bring the volume to one liter with Deionized Water. Mix well.

10.3N POTASSIUM HYDROXIDE

670 gm of Potassium Hydroxide (Assay 88%) is added slowly with cooling and mixing into 400 mL of Deionized Water. When all the solid is dissolved and the solution is at room temperature bring the volume to one liter with water.

SODIUM CARBONATE/BICARBONATE BUFFER, pH 9.2

Prepare a saturated solution of Sodium Bicarbonate (60 gm/ liter Deionized Water). pH should be 8.4. Raise the pH to 9.2 with Anhydrous Sodium Carbonate. Decant the clear supernatant solution and discard the solid at the bottom.

SATURATED POTASSIUM PHOSPHATE, MONOBASIC

Add 10 grams of Potassium Phosphate Monobasic to 100 mL Deionized Water. Mix will.

SATURATED SODIUM BISULFITE SOLUTION

Dissolve 25 grams of Sodium Bisulfite (or Sodium Metabisulfite which converts to Sodium Bisulfite in solution) in 50 mL of Deionized water. Shake well. Dilute 1:1 with Reagent D, Phosphate Buffer, pH 6.0 for a total volume of 100mL. Shake well. Prepare monthly and store this reagent in a glass bottle with a Teflon or Polypropylene lined screw capped top. [See](#)

[Package Insert](#)

TRIS BUFFER, pH 9.0

Dissolve 12.1 grams of TRIS (THAM) base in 900 mL of Deionized Water. Adjust the pH to 9.0 with the slow addition of concentrated HCl.

SOLVENTS

Precautions:

All solvents should be HPLC grade.

Because some drugs are changed when exposed to an oxidizing agent it is important that Ethyl Acetate (which easily forms peroxides) be peroxide free. Check for peroxides by mixing 1 ml of a 50% (w/v) of Potassium Iodide in water with 9 mL of solvent. A brown color indicates release of Iodine by the oxidative effect of organic peroxides. A drop or two of 0.1 N HCl added to the tube increases the sensitivity of the test.

It is a good idea to screen each new lot # of solvents for contaminants by drying down a few mL of the solvent and following derivatization, if required, injecting it for GC-MS analysis. As an example, we have encountered a contaminant in Acetone which, when used to prepare the 40% wash for THC sample preparation, resulted in a 488 ion contamination at the retention time of THC-COOH. A different lot # of Acetone did not exhibit this contaminant.

Solvent Preparation

Ethyl Acetate: Hexane (1:1, v/v), Acidified with 2 % Glacial Acetic Acid (2 mL Glacial Acetic Acid in 98 mL mixed solvent). Shake well. Prepare monthly and store this reagent in a glass bottle with a Teflon or Polypropylene lined screw capped top.

Solvents or solvent mixes which call for the addition of 2% Triethylamine (TEA) are prepared in a glass bottle with a Teflon or Polypropylene lined screw capped top. 2 ml of TEA is added to 98 mL of the appropriate solvent and mixed well. Prepare fresh reagent bi-monthly.