



INTERFINE CHEMICALS ANALYTICAL SCIENCES BIOCHROMATOGRAPHY LIFE SCIENCES

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## Water Saturated Phenol Solutions

*Molecular Biology grade for nucleic acid purifications*

### Product Description

|  |          |        |
|--|----------|--------|
| <b>Saturated Phenol pH 6.6 / &amp; final pH 7.9*</b>   | UP87336A | 100 ml |
|  | UP87336B | 400 ml |
| *after addition of buffer. A bottle of buffer is included for most DNA applications, that require a higher pH of $7.9 \pm 0.2$ . |          |        |
| <b>Saturated Phenol pH <math>4.3 \pm 0.2</math></b><br>for use in RNA purifications  | UP893305 | 100 ml |
|  | UP893306 | 400 ml |
| <b>Phenol / Chloroform / Isoamyl alcohol 25:24:1 pH 6.7 / for pHf 8.0*</b>   | UP873357 | 100ml  |
|  | UP873359 | 400ml  |
| *after addition of buffer. A bottle of buffer is included for most DNA applications, that require a higher pH of $8.0 \pm 0.2$ . |          |        |
| <b>Phenol / Chloroform / Isoamyl alcohol 25:24:1 pH 5.2</b>  | 57442A   | 100ml  |
|  | 57442B   | 400ml  |

Storage: Store cold. Warm to room temperature before opening.

### Technical Information

Preparing phenol in a form suitable for applications in molecular biology is a time consuming and often-hazardous procedure. First, the phenol must be distilled under nitrogen and then stored frozen to prevent deterioration. It has a very limited shelf life and often becomes unsuitable for use and must be discarded. Next, the phenol must be melted and saturated with buffer before it can be used. Finally, the pH should be adjusted depending on applications.

**Uptima** Saturated Phenols eliminate these handling difficulties and are available in three pH's for use in different applications.

All our Saturated Phenols are prepared from high purity phenol (>99%), double distilled under nitrogen. This material is then saturated completely, and extra buffer is added as an overlay to prevent oxidation. The products are packaged under nitrogen for maximum stability and remains stable for greater than one year when stored unopened at 4°C.

Before use, phenol must be buffered to pH greater than 7.8 because DNA partitions into the organic phase at acid pH and the DNA would then be lost.



Additives:

The **chloroform** denatures the proteins and facilitates the separation of the aqueous and organic phases.  
The **isoamyl alcohol** reduces foaming during the extraction process.

Stability:

Liquefied **phenol** is colorless, once it takes on a pink or yellow tinge it should be rejected.

A **Tris** solution is provided to adjust precisely pH to desired application (see protocol 1).

Phenol oxidizes much quicker at higher pH values and will turn red after a period of several months, once it does this it cannot be used. The oxidation is speeded up at higher pH 8.0. Lower pH eg. pH 6 or freezing the solution can increase the shelf life (~1 year).

The pH can be checked using a pH meter but we recommend the method below as phenol solutions are very aggressive. Once the pH is established then repeating the volumes used should give the correct pH each time.

**Protocol 1 – Phenol solution preparation: pH adjustment and measurement** <sup>[r]</sup>

WEAR GLOVES, LABCOAT AND WORK IN A FUME HOOD.

● 1a-Saturated Phenol pH adjustment

Add the suitable quantity of the Tris buffer to the equilibration buffer of Phenol bottle. Mix gently and allow the phases to separate before use, approximately 2-4 hours. Check for pH (step 1c or 1d).

● 1b-Measure pH for phenol: chloroform: IAA or Acid phenol: chloroform Solutions

Mix 2 ml of the phenol organic phase\* with 8ml of Methanol.

Then add 10ml of purified water.

Measure the pH of the entire solution using a reference electrode (pH Probe).

● 1c-Measure pH for Water Saturated Phenols

Mix 2 ml of the phenol organic phase\* with 5ml of Methanol.

Then add 13ml of purified water

Measure the pH of the entire solution using a reference electrode (pH Probe).

\*When taking the reagent you must pipette from beneath the aqueous layer.

This pH-adjusted phenol can be used to determine the quantity of Saturated Tris-buffer to use in order to adjust the pH of solution prior to use or aliquoting into 1 ml micro-tubes for use in extraction.



**Protocol 2 – Phenol Chloroform Extraction of DNA** <sup>[1]</sup>

WEAR GLOVES, LABCOAT AND WORK IN A FUME HOOD.

Dispose of all waste as hazardous waste.

If any phenol spills onto the skin it should be washed of with PEG, use water if PEG is not available.

Actual volumes are not given – this protocol is dependent on the amount of starting material.

1. Add the DNA sample and an equal volume of Phenol:Chloroform:Isoamyl Alcohol (25:24:1)  
When taking the Phenol reagent you must pipette from beneath the buffer layer.
2. Vortex for a few seconds to mix well and form an emulsion.
3. Centrifuge at RT for 5min at 12-14K.
4. Pipette off and keep the top aqueous phase  
– you MUST avoid taking any precipitated material from the interphase or any phenol.
5. Add 1/10th volume 3M sodium acetate pH 5.5
6. Add 2 volumes of 100% ethanol
7. Mix slowly by inversion – the DNA may be seen to precipitate out of solution as a “ball of fluff”.
8. If possible spool the DNA out of the tube.
9. If the DNA does not spool ensure the sample is mixed well – vortex if it is not genomic DNA.
10. Centrifuge at RT for 15min at 12-14K.
11. Wash with 70% ethanol – make sure all the ethanol is removed – re-centrifuge if necessary.
12. Air dry briefly but do not overdry.
13. Resuspend as required

## Ordering information

Catalog size quantities and prices may be found at <http://www.interchim.com>.

Please inquire for higher quantities (availability, shipment conditions).

For any information, please ask : Uptima / Interchim; Hotline : +33(0)4 70 03 73 06

[Order on-line](#) or [Contact](#) your local distributor

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