

NaPi/PEG hybridization buffer

Prepare phosphate buffer as follows:

approx. 500 mL demineralised water in 2 Liter beaker

Dissolve 44,45 gram $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ (MW=177,99, Merck, Fluka)

Dissolving takes 1 hour, heat a little bit, not more than 40°C

Set pH with 85% phosphoric acid (2-3 mL) to exactly pH = 7.2

Do not sterilize, prepare previous to preparation hybridization buffer.

Add to phosphate buffer:

100 mL NaCl (5M)

4 mL 0.5 M EDTA (pH 8.0)

140 gram SDS, or 700 mL 20% SDS solution (for solid SDS use the hood (very toxic))

200 gram PEG-6000

Add until almost 2 Liter with demineralised water

Heat in water bath or microwave until 65°C, than stir until PEG and SDS are dissolved.

Finally add demineralised water until exactly 2 Liter.

For hybridization:

Heat until 65°C

Add 100 ug/ml Salmon sperm DNA to hybridization buffer (preheated at 95°C) and start prehybridization (standard prehybridization at least 45 minutes)

We do not replace the 'pre'-hybridization buffer by fresh hybridization buffer.

2xNaPi hybridization buffer (for Formamide hyb mix)

Prepare 2x phosphate buffer as follows:

approx. 500 mL demineralised water in 2 Liter beaker

Dissolve 44,45 gram $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ (MW=177,99, Merck, Fluka)

Dissolving takes 1 hour, heat a little bit, not more than 40°C

Set pH with 85% phosphoric acid (2-3 mL) to exactly pH = 7.2

Do not sterilize, prepare previous to preparation hybridization buffer.

Add to phosphate buffer:

100 mL NaCl

4 mL 0.5 M EDTA (pH 8.0)

140 gram SDS, or 700 mL 20% SDS solution (For solid SDS use the hood (very toxic))

Add until almost 1 Liter with demineralised water

Heat in water bath or microwave until 65°C, than stir until SDS is dissolved.

For hybridization:

Heat until 65°C

Add 1 volume of deionized Formamide

Add 100 ug/ml Salmon sperm DNA to hybridization buffer (preheated at 95°C) and start prehybridization (standard prehybridization at least 45 minutes)

We do not replace the 'pre'-hybridization buffer by fresh hybridization buffer.