**Neutralization Buffer B**

*(For transfer to nylon membranes)*

**Introduction:** Neutralization Buffer B is mainly used during Southern blotting procedure where DNA is transferred from agarose gel to positively charged nylon membranes. This buffer lowers the pH of DNA during the blotting process and comes as a ready to use solution and can be directly used.

**Description:** Southern blotting has been one of the foundation of DNA analysis since its first description by E.M. Southern in 1975. Originally nitrocellulose membranes were used but their fragility prompted to search for alternative types of support matrix and as a result nylon membranes were implemented. Neutralization Buffer B is used during Southern hybridization to neutralize DNA agarose gels after the DNA denaturation before transfer to nylon membranes. During denaturation step double stranded DNAs are unzipped to give single-stranded DNAs which are suitable for hybridization. The main objective of neutralization solution is to bring pH below 9 as at higher pH, DNA does not bind to the membrane.

**Application:** Neutralization Buffer B is mainly used to neutralize DNA agarose gels after the DNA denaturation in base step before transfer to nylon membranes.

**Composition:** Neutralization Buffer B is composed of 0.5 M Tris and 1 M Sodium chloride and the pH is adjusted to 7.2.

**Properties:**

- **Appearance**: Colorless solution
- **Clarity**: Clear and free of particles
- **pH**: 7.1 - 7.3
- **DNase & RNase**: None detected
- **Bioburden**: None detected
- **Suitability test**: This solution has been tested and is suitable for use

**Storage conditions:** Neutralization Buffer B has to be stored at room temperature (15 - 25°C).

**Technical Assistance**

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