HiPurA® Fast Southern Hybridization Buffer

Product Name	Product Code	Kit Packing
HiPurA® Fast Southern Hybridization Buffer	ML209-50 ML	50 ML
	ML209-125 ML	125 ML
	ML209- 1 L	1 L

Intended Use:

Recommended for DNA hybridization in Southern blot protocols using biotinylated probes.

Introduction:

HiMedia's Fast Southern Hybridization Buffer is a ready-to-use hybridization buffer for Southern blot protocols. It has been optimized to achieve maximum DNA hybridization within 3-4 hrs with less background.

Description:

Southern blotting is a commonly used method for identification of DNA that are complementary to a known DNA sequence. It allows a comparison between the genome of a particular organism and that of an available gene or gene fragment. This technique confirms whether an organism contains a particular gene and provides information about the organization and restriction map of that gene.

In southern blotting, conventional Buffers take 16 hours of hybridization time. While, Himedia's Fast Southern Hybridization Buffer reduces incubation time from 16 hours to 2-3 hours. It enhances signal intensity and reduces background. HiPurA® Fast Southern Hybridization Buffer works at 68^0C and reduced time of hybridization i.e., 2-3 hours. It follows a similar protocol as conventional hybridization buffers, only the temperature and time of hybridization is different. This buffer doesn't require blocking step. To achieve maximum sensitivity, time of hybridization can be extended. This increases band intensity without increasing background level. This buffer is compatible for non-radioactive detection where probe is labelled with biotin.

Application:

It has been designed to work in any DNA hybridization protocols, using any type of probe (single or double-stranded DNA, RNA, and oligonucleotide probes) and on any type of membrane (nylon or nitrocellulose).

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Properties:

Appearance/ Color/ Clarity	: Colorless to Yellowish clear solution and free of particles
DNase & RNase	: None detected
Suitability Test	: This solution has been tested and is suitable for use in nucleic acid hybridization procedures.

Storage Conditions:

HiPurA® Fast Southern Hybridization Buffer can be stored at 2-8°C. The reagent is filter sterilized and should be open under aseptic conditions. If precipitate forms, heat the solution to 60°C and mix until it get dissolved.

Materials needed but not provided:

- 20X SSC(Product Code: ML030)
- 20% SDS (ML007)
- Molecular Biology Grade Water (Product Code: ML064)
- HRP-streptavidin Conjugate Buffer

Preparation Instructions:

- **Low Precision Wash Buffer** (2X SSC, 0.1% SDS)
  In 70 ml of molecular biology grade water, add 10 ml of 20X SSC and 0.5 ml of 20 % SDS. Make up the volume to 100 ml with water.
- **High Precision Wash Buffer** (0.5X SSC, 0.1% SDS)
  In 80 ml of molecular biology grade water, add 2.5 ml of 20X SSC and 0.5 ml of 20 % SDS. Make up the volume to 100 ml with water.
- **Super-High Precision Wash Buffer** (0.1X SSC, 0.1% SDS)
  In 90 ml of molecular biology grade water, add 0.5 ml of 20X SSC and 0.5 ml of 20% SDS. Make up the volume to 100 ml with water.

Protocol :

1. Pre-hybridize membranes in 10 ml of **HiPurA® Fast Southern Hybridization buffer** for at least 5 minutes at 68^0C with a mild shaking at 70-90 rpm.

Probe Type	Hybridization Temperature
DNA	68^0C
RNA	68^0C
Oligonucleotides	37-45^0C

NOTE: Membranes should be completely covered with sufficient volume of hybridization buffer.

2. For denaturation of biotinylated double stranded DNA probes, keep 1 vial of probe in boiling water bath for 10 minutes and immediately chill on ice for 10 minutes.
   NOTE: Probe should be properly labelled and its efficiency should be checked prior to use. The concentration of the probe should be 1-10 ng/ml. Higher concentration of probe tends to generate background signal.

3. Add denatured 15 µl of probe to the hybridization buffer in the petriplate or replace Prehybridization buffer with fresh prewarmed **HiPurA® Fast Southern Hybridization Buffer**.
4. Incubate membranes in 10 ml of HiPurA® Fast Southern Hybridization Buffer for 2-3 hours of incubation at 68^0C incubator shaker with mild shaking at about 70-90 rpm. For maximum sensitivity, length of hybridization can be extended.
5. After hybridization is complete, discard the buffer. Wash membrane 2 x 5 min in 10 ml of **low precision wash buffer** at room temperature. Gently swirl the petri-plate. Discard the buffer after each wash.
6. Add 10 ml of prewarmed **high precision wash buffer** and gently swirl the petri-plate for 2 x 15 minutes at 68^0C. Discard the buffer after each wash.
7. Add 9 ml of diluted **streptavidin-HRP conjugate buffer** to the petriplate and incubate at room temperature for 20 minutes with gentle rocking. Discard the conjugate buffer.
8. For highest precision, a final wash for 2 x 15 minutes with **super-high precision wash buffer** is added. Discard the buffer after each wash.
9. Add 5 ml of **TMB/H_2O_2** and gently swirl at room temperature for 5 minutes until a blue colour band develops.
10. After blue colour is seen stop the reaction by placing the membrane in distilled water.

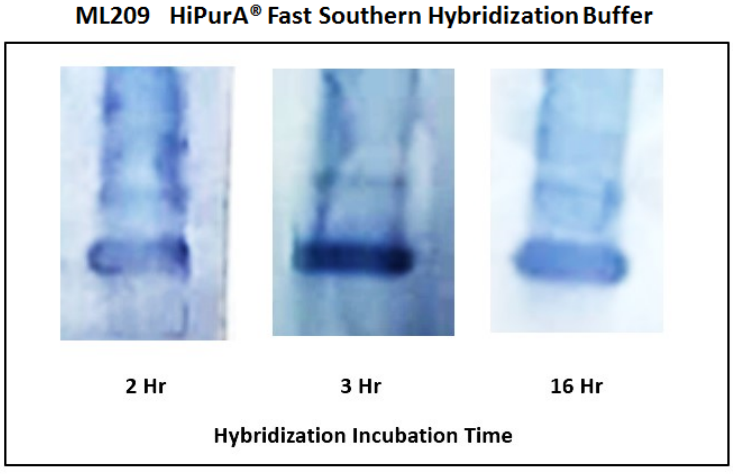


Fig 1: Data of HiPurA® Fast Southern Hybridization Buffer

Limitation:

The protocols for a specific application can vary. Appropriate probe concentration and protocol parameters may depend upon multiple factors and must be empirically determined by the user.

Troubleshooting Guide:

Problem	Cause	Solution
Low Sensitivity (Faint bands or no bands)	Incomplete transfer	Following DNA transfer, view the membranes under UV transilluminator to check for proper and complete DNA transfer.
	Target DNA not effectively fixed on membrane	Check UV lamp or baking temperature.
High Background	Insufficient washing or contamination in buffer	Wash the membranes thoroughly as mentioned in the brochure.
	Probe added onto membrane	Always add the probe to hybridization solution far away from the membrane.
	Probe concentration was too high	Decrease probe concentration. High-concentration probes tend to generate high background signals.
	Drying of the membrane at hybridization steps	Drying of the membrane at the hybridization step increases background signals. Take care that the membrane does not become dry.
	Concentration of enzyme conjugate is too high.	Dilute the enzyme conjugate further. The specific dilution required for optimal signal to noise must be determined empirically.
Weak/Absent Signal	Target nucleic acids are not present, have been degraded, or are too low for detection.	Run agarose gel electrophoresis to confirm nucleic acids are not degraded. Load more target nucleic acids for blotting. For Southern blots, up to 10 µg DNA can be loaded per lane.
	Probe was not labelled efficiently	For non-radioactive probes, check the incorporation of Biotin by spotting and detecting serial dilutions of probe in direct comparison to a known standard. If probes are not labeled well enough, remake and confirm adequate incorporation rates.
	Detection system is not working properly	Using serial dilutions of the enzyme/antibody conjugate confirm the functionality by spotting and detecting the labeled probe on membrane.

Warning and Precautions

Not for Medicinal Use. Read the SDS carefully before beginning the protocol. Wear protective gloves/protective clothing/eye protection/face protection. Follow good clinical laboratory practices while handling clinical samples. Standard precautions should be followed as per established guidelines. Safety guidelines may be referred in safety data sheets of the product.

Please refer disclaimer Overleaf.

Performance and Evaluation

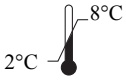
Performance of the buffer is expected when the buffer is stored at recommended temperature and within the expiry period.

Safety Information

The HiPurA® Fast Southern Hybridization Buffer is for laboratory use only, not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves and safety goggles when handling. Not compatible with disinfecting agents containing bleach. Please refer the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.

Technical Assistance

At HiMedia we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance, mail at mb@himedialabs.com.



Storage temperature

Do not use if package is damaged

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