

# Amersham Microarray Hybridization Buffer

## Product Specification Sheet

### Introduction

#### Product code

RPK0325

#### About

Proprietary microarray hybridization buffer - ensures low background and increased signal to noise ratio in hybridization.

#### Important

Read these instructions carefully before using the products.

#### Intended use

The products are intended for research use only, and shall not be used in any clinical or *in vitro* procedures for diagnostic purposes.

#### Safety

For use and handling of the products in a safe way, refer to the Safety Data Sheets.

#### Storage

Hybridization buffer should be stored at -15°C to -30°C. Do not store in a frost free freezer.

#### Expiry

For expiry please refer to outer packaging.

#### Component

Microarray hybridization buffer RPK0325, 2 x 1 mL.

### Other materials required

Formamide, deionized, RNase-free, DNase-free: for example, Ambion Product code 9342 A<sub>80</sub>, synthesized oligonucleotide.

### Protocol

#### Pre-hybridization

Step	Action
1	Pre-hybridize with 5 x SSC, 0.2% SDS, 1% BSA at 60°C for 20 minutes.
2	Proceed with the next part of the protocol.

#### Hybridization

Step	Action
1	Dilute probe in H <sub>2</sub> O, heat to 94°C for 3 minutes to denature, transfer immediately to ice.

Step	Action												
2	Add A <sub>80</sub> at 1 µg per slide to a total volume of 7.5 µL.												
3	Incubate at 70°C for 45 minutes and then at room temperature for 30 minutes.												
4	Prepare the final hybridization mixture as set out in the table below:												
	<table border="1"> <thead> <tr> <th></th> <th>Volume (µL)</th> <th>Final concentration</th> </tr> </thead> <tbody> <tr> <td>A<sub>80</sub> treated probe</td> <td>7.5</td> <td>as required</td> </tr> <tr> <td>Hybridization buffer (RPK0325)</td> <td>7.5</td> <td>1 x</td> </tr> <tr> <td>Formamide</td> <td>15</td> <td>50% (v/v)</td> </tr> </tbody> </table>		Volume (µL)	Final concentration	A <sub>80</sub> treated probe	7.5	as required	Hybridization buffer (RPK0325)	7.5	1 x	Formamide	15	50% (v/v)
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A <sub>80</sub> treated probe	7.5	as required											
Hybridization buffer (RPK0325)	7.5	1 x											
Formamide	15	50% (v/v)											
5	Hybridize at 42°C under a coverslip in a humid chamber, overnight.												
6	Proceed with the next part of the protocol.												

#### Post-hybridization washes

Step	Action
1	Dip the slide in pre-warmed 1 x SSC, 0.1% SDS to remove the coverslip.
2	Wash the slide using the following conditions: 1 x SSC, 0.1% SDS, at 55°C for 5 minutes 0.1% SSC, 0.1% SDS at 55°C for 10 minutes, twice 0.01 x SSC, 0.1% SDS at 55°C for 10 minutes.

### Additional information

#### Microarray reagents

The CyDye™ fluors from Cytiva have gained world-wide acceptance as exceptional dyes for microarray detection. Cy™3 and Cy5 have become the labels of choice for this application because they offer bright and intense colours with narrow emission bands.

These two dyes are spectrally distinct, making them ideal for dual colour detection in microarrays.

**Bright, intense dyes** - CyDye fluors give bright signals increasing the amount of information that can be gained from microarray analysis.

**High sensitivity** - Meaningful data can be generated from low copy number transcripts.

**Spectrally resolved dyes** - for multiplexing, give discreet, discernible signals. The CyDye absorption and emission spectra are sharp, resulting in good spectral separation of the different fluors on the same microarray slide.

Their other properties are:

- low non-specific binding
- high photo-stability
- high water solubility
- pH insensitivity
- survival in harsh hybridization conditions

CyDye fluors are available as CyDye-deoxynucleotides, CyDye ribonucleotides and highly reactive NHS Esters.

## Related products

CyDye Post-Labeling Reactive Dye Pack, 12 of Cy3 and 12 of Cy5, 24 reactions RPN5661

### CyDye nucleotides

Cy3 dCTP	25 nmol	PA53021
Cy5 dCTP	25 nmol	PA55021
Cy3 dUTP	25 nmol	PA53022
Cy5 dUTP	25 nmol	PA55022
Cy3.5 dCTP	25 nmol	PA53521
Cy5.5 dCTP	25 nmol	PA55521

### CyDye value packs

Cy3dCTP 25 nmol x 5 plus	
Cy5dCTP 25 nmol x 5	PA55321
Cy3 dUTP 25 nmol x 5 plus	
Cy5 dUTP 25 nmol x 5	PA55322

Custom CyDye nucleotide packs are available on request.

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