Amersham HyPer5 Dye

Amersham HyPer™5 dye is a new fluorescent dye for microarray applications. It combines exceptional ozone and photostability with highly reproducible performance all year round—irrespective of environmental conditions. HyPer5 dye is available in two formats: first, as a HyPer5 NHS reactive dye for coupling to aminoallyl modified-cDNA or amplified RNA in post-labeling experiments. Second, as a HyPer5-dCTP for direct enzymatic incorporation and probe labeling reactions. HyPer5 dye allows you to perform microarray experiments using existing labeling methods and filter settings on imaging instruments.

Amersham HyPer5 dye offers:

- **Robustness and environmental stability:** Photostability and resistance to signal loss from exposure to light, ozone, and repeated scanning, thereby providing highly consistent and reproducible results
- **Ozone stability:** Three-fold more ozone stability than Alexa Fluor™ 647 enables array experiments to be performed with HyPer5 dye under any environmental condition
- **Photostability:** 50% more photostability than Alexa Fluor 647 and this prevents a decline in signal strength from the rescanning of arrays
- **Efficient probe labeling:** Probes can be generated by a post-labeling method or direct incorporation starting from total and mRNA for gene expression experiments. In addition, the ability to synthesize probes from genomic DNA for array CGH applications demonstrates the versatility of HyPer5 dyes
- **Dual-color hybridization:** Allows direct comparison of HyPer5 dye signal levels with Cy™3 in expression analysis from both direct- and post-labeling experiments

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### Table 1. Comparative evaluation of HyPer5 and Alexa Fluor 647 dyes

<table>
<thead>
<tr>
<th>Experiment</th>
<th>HyPer5 dye</th>
<th>Alexa Fluor 647 dye</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rescanning</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Photostability (Absorbance after 7 days)</td>
<td>94%</td>
<td>50%</td>
</tr>
<tr>
<td>Ozone resistance (Signal remaining after 100 ppb ozone exposure)</td>
<td>+++</td>
<td>+ 29%</td>
</tr>
<tr>
<td>Labeling NHS probe yield</td>
<td>+++</td>
<td>+ 87 pmoles</td>
</tr>
<tr>
<td>Labeling dCTP probe yields</td>
<td>++</td>
<td>++ 26 pmoles</td>
</tr>
</tbody>
</table>

+++ = best performance; ++ = good performance; + = poor performance

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### Functional evaluation of HyPer5 Dye

#### Photostability

The photostability of HyPer5 dye was compared to Alexa Fluor 647, Cy5, and Cy3 dyes by repeated scanning of a hybridized array. HyPer5 probes synthesized from total RNA spiked with Universal ScoreCard control RNA were hybridized to a zebrafish array printed with Universal ScoreCard genes. The signals from scorecard control genes were quantitated and measured after repeated scanning of the arrays. HyPer5 dye signals remained constant upon array rescanning (Fig 1); emphasizing the photostability of HyPer5 dyes.
The graph shows signal strength from an average of 10 scorecard control genes after repeated scanning of microarrays hybridized with different dyes. Cy5 and Alexa Fluor 647 probes were produced with the Amersham CyScribe First-Strand cDNA Labeling Kit and SuperScript™ Indirect cDNA Labeling System, respectively. The microarrays were scanned with ScanArray™ at a maximum laser power of 633 nm for 4 min with a 2 min interval between scans. The signal from HyPer5 dyes declined by 6% after five scans whereas that of Alexa Fluor 647 declined by 17%. Data kindly provided by Dr. Peter Kille, Cardiff University.

At the beginning of the second scan, the signal strength for HyPer5 dye remained constant unlike that for Cy5 and Alexa Fluor 647 which suffered a significant decline. After the fifth scan, the HyPer5 dye signal was 94% compared to 78% and 83% for the Cy5 and Alexa Fluor 647, respectively. The photostability of HyPer5 was comparable to Cy3 which showed negligible signal loss from control genes after five scans.

For photostability determination, HyPer5 dye and Alexa Fluor 647 were exposed to an incandescent light source for 7 d. HyPer5 dye showed negligible loss in absorbance value; unlike the Alexa Fluor 647 which suffered about a 50% loss in absorbance (Fig 2). This shows that HyPer5 dye is more photostable than the Alexa Fluor 647.

The exposure of hybridized microarrays to high concentrations of ozone reduces Cy5 and Alexa Fluor 647 signals and distorts gene expression ratio analysis (3). Ozone levels greater than 25 ppb inside laboratories are known to severely impact Cy5 and Alexa Fluor 647 array signals from dye degradation (3). The sensitivity of HyPer5, Cy5, and Alexa Fluor 647 to ozone was measured by exposing hybridized zebrafish cDNA array to 100 ppb ozone for 5 min (using an ozone generator) followed by scanning and quantitation of signal levels from Universal ScoreCard control genes (Fig 3). Even after two ozone treatments at the elevated levels of 100 ppb, the HyPer5 dye signal was completely intact on microarray, whereas, Cy5 and Alexa Fluor 647 signals dropped to 25% and 29%, respectively. HyPer5 dye offers reliable performance even under elevated ozone conditions.

For labeling efficiency, HyPer5 NHS reactive dye was used in coupling reactions with both total and mRNA templates according to the protocol outlined in the Amersham CyScribe™ Post-Labeling Kit. With an input template amount of 20 μg of total RNA, we observed that the efficiency of HyPer5 labeling was higher than that of Cy5 NHS reactive dye and 7-fold higher than that of Alexa Fluor 647. Thus, HyPer5 NHS reactive dye demonstrates high coupling efficiency and faster reaction times than Cy5 NHS and Alexa Fluor 647 NHS reactive dyes.
When mRNA (1 μg) was used in post-labeling reactions, the HyPer5 NHS reactive dye showed better coupling efficiency than Cy5 NHS (Table 3).

Table 3. Post-labeling data starting from 1 μg of mouse mRNA with HyPer5- and Cy5-NHS reactive dyes.

<table>
<thead>
<tr>
<th></th>
<th>HyPer5 NHS reactive dye</th>
<th>Cy5 NHS reactive dye</th>
<th>Alexa Fluor 647 NHS reactive dye</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dye incorporation (pmol)</td>
<td>87.1</td>
<td>34.5</td>
<td>12.4</td>
</tr>
<tr>
<td>Nucleotide per dye ratio</td>
<td>29</td>
<td>77</td>
<td>354</td>
</tr>
</tbody>
</table>

mRNA templates produced a greater yield of probes than total RNA input. HyPer5 NHS reactive dye can be used to label aminoallyl-modified amplified RNA (cRNA) synthesized by Eberwine amplification reactions for hybridization on oligonucleotide-based slides. Table 4 shows a typical labeling reaction starting with 500 ng of human universal RNA. About 6 μg of aminoallyl cRNA was generated and 2 μg of this was used per labeling reaction.

Table 4. Aminoallyl cRNA labeling data with HyPer5 NHS.

<table>
<thead>
<tr>
<th></th>
<th>HyPer5 NHS reactive dye</th>
<th>Cy5 NHS reactive dye</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nucleotide concentration (ng)</td>
<td>1229.41</td>
<td>752.94</td>
</tr>
<tr>
<td>Dye incorporation (pmol)</td>
<td>26.4</td>
<td>10.2</td>
</tr>
<tr>
<td>Nucleotide per dye ratio</td>
<td>47</td>
<td>74</td>
</tr>
</tbody>
</table>

**Functional evaluation of HyPer5-dCTP**

HyPer5-dCTP can be readily incorporated into cDNA using the direct incorporation method. The enzymatic incorporation of HyPer5-dCTP was found to be equal to Cy5-dCTP in conventional first-strand synthesis reactions starting from both total and mRNA (Tables 5 and 6).

Table 5. Labeling data using the direct incorporation method using 1 μg of mouse mRNA with HyPer5- and Cy5-dCTP with the Amersham CyScribe First-Strand cDNA Labeling Kit. The data represents the average of a sample size of eight (n = 8) and ten (n = 10) HyPer5- and Cy5-dCTP reactions, respectively. For each reaction, 1 μl of HyPer5- and Cy5-dCTP was added to the labeling reactions.

<table>
<thead>
<tr>
<th></th>
<th>HyPer5-dCTP</th>
<th>Cy5-dCTP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dye incorporation (pmol)</td>
<td>44.7</td>
<td>43.2</td>
</tr>
<tr>
<td>Nucleotide per dye ratio</td>
<td>42</td>
<td>38</td>
</tr>
</tbody>
</table>

We synthesized 40 to 60 pmoles of HyPer5-labeled cDNA probes from 1 μg of mRNA template and 1 μl of HyPer5-dCTP. For total RNA, input template amounts of 20 μg or more produced 25 to 35 pmoles of labeled cDNA per labeling reaction (Table 6).

Table 6. Labeling data using the direct incorporation method starting from 20 μg of mouse total RNA with HyPer5- and Cy5-dCTP using the Amersham CyScribe First-Strand cDNA Labeling Kit. Each value reported represents the average of three measurements.

<table>
<thead>
<tr>
<th></th>
<th>HyPer5-dCTP</th>
<th>Cy5-dCTP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dye incorporation (pmol)</td>
<td>32</td>
<td>35</td>
</tr>
<tr>
<td>Nucleotide per dye ratio</td>
<td>94</td>
<td>70</td>
</tr>
</tbody>
</table>

To achieve efficient dye incorporation during direct incorporation reactions, we recommend a HyPer5-dCTP to dCTP ratio of 3:1. Depending on the reverse transcriptase enzyme or kit used, you may have to optimize the labeling reaction by titrating the amount of HyPer5-dCTP per reaction.

**HyPer5 dye microarray gene expression data**

HyPer5 dye probes can be hybridized onto microarrays using existing CyDye™ hybridization protocols and array buffers. The conditions employed for post-hybridization washes are also identical to those used with Cy5 dyes. We recommend that you hybridize a minimum of 50 pmol of HyPer5 labeled probes onto each array.

HyPer5 dye performs similarly to that of Cy3 in gene expression experiments. mRNA was labeled with HyPer5-dCTP and hybridized onto microarray containing 3000 genes (printed in triplicates) and the results (Table 7) show that the average signal for all genes was similar for both HyPer5 and Cy5 dyes.

Table 7. Hybridization data from microarray with 3000 genes (in triplicates) hybridized with HyPer5/Cy3 and Cy5/Cy3. Data kindly provided by Dr. Peter Kille, Cardiff University.

<table>
<thead>
<tr>
<th></th>
<th>Cy5 or HyPer5 average signal</th>
<th>Cy3 average signal</th>
<th>Count of genes above background</th>
</tr>
</thead>
<tbody>
<tr>
<td>HyPer5 array</td>
<td>2383</td>
<td>1847</td>
<td>4574</td>
</tr>
<tr>
<td>Cy5 array</td>
<td>2522</td>
<td>2292</td>
<td>4598</td>
</tr>
</tbody>
</table>

Equivalent performance between HyPer5 NHS reactive dye and Cy5 NHS reactive dye was also obtained. Mouse mRNA was labeled with both dyes using the Amersham CyScribe Post-Labeling Kit and the probes were hybridized onto arrays containing 11 424 features (Table 8). HyPer5 array signal depends on the amount of labeled probe used. Therefore, it is possible to increase the signal from HyPer5 by increasing the amount of labeled probe in hybridization.
Summary

HyPer5 dye can be readily incorporated to generate labeled probes from both total and mRNA with improved efficiencies. For microarray hybridization, HyPer5 dye demonstrated vastly increased ozone and photostability relative to Alexa Fluor 647. Array signals from HyPer5 dyes were found to be resistant to elevated ozone levels and repeated scanning of slides. The photostability of HyPer5 dyes was 50% more than that for Alexa Fluor 647. Furthermore, microarray hybridization with HyPer5 allows gene expression levels to be compared with Cy3 on the same arrays. HyPer5 dyes allow microarray experiments to be performed under any environmental condition using existing labeling methods and filter settings on imaging instruments.

References


Table 8. Hybridization data from mouse array comparing HyPer5 NHS to Cy5 NHS.

<table>
<thead>
<tr>
<th>Dye</th>
<th>Cy5 or HyPer5 average signal</th>
<th>Cy5 average signal</th>
<th>Count of genes above background</th>
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</thead>
<tbody>
<tr>
<td>HyPer5 array</td>
<td>471</td>
<td>937</td>
<td>5458</td>
</tr>
<tr>
<td>Cy5 array</td>
<td>540</td>
<td>927</td>
<td>5858</td>
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Ordering information

<table>
<thead>
<tr>
<th>Product</th>
<th>Quantity</th>
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<tbody>
<tr>
<td>HyPer5 dCTP</td>
<td>25 nmol</td>
<td>28-9231-83</td>
</tr>
<tr>
<td>HyPer5 dCTP</td>
<td>250 nmol</td>
<td>28-9231-84</td>
</tr>
<tr>
<td>Multi pack containing 5 ×</td>
<td></td>
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<tr>
<td>25 nmol Cy3 dCTP + 5 ×</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25 nmol HyPer5 dCTP</td>
<td></td>
<td></td>
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<tr>
<td>Cy3 and HyPer5 Post-</td>
<td>1 pack</td>
<td>28-9224-19</td>
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<tr>
<td>Labeling Reactive Dye Pack (12 × 40 000 pmol</td>
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<tr>
<td>Cy3 + 12 × 15 000 pmol</td>
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<tr>
<td>HyPer5)</td>
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<tr>
<td>HyPer5 Post-Labeling</td>
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<td>Reactive Dye Pack</td>
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Related products

Amersham CyDye Fluorescent Nucleotides
Amersham CyDye Value Packs (mono-Reactive NHS Ester)
Amersham CyScribe First-Strand cDNA Labeling Kit
Amersham CyScribe Post-Labeling Kit
Amersham CyScribe Array CGH Genomic Labeling System
illustra™ CyScribe GFX Purification Kit
illustra GFX™ PCR DNA and Gel Band Purification Kit
illustra GenomiPhi V2 DNA Amplification Kit

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www.gelifesciences.com/hyperdye

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