

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

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

Experiment	HyPer5 dye	Alexa Fluor 647 dye
Rescanning	+++	+
Photostability (Absorbance after 7 days)	+++ 94%	++ 50%
Ozone resistance (Signal remaining after 100 ppb ozone exposure)	+++ 100%	+ 29%
Labeling NHS probe yield	+++ 87 pmoles	+ 12 pmoles
Labeling dCTP probe yields	++ 26 pmoles	++ 26 pmoles

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

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.



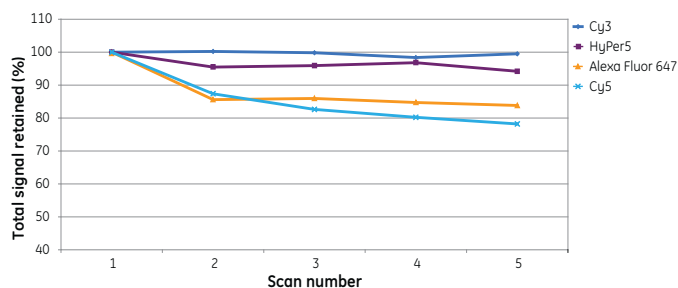


Fig 1. The graph shows signal strength from an average of 10 scorecard control genes after repeated scanning of microarrays hybridized with different dyes. Cy5 and Alexa Fluor 647 probes were produced with the Amersham CyScribe First-Strand cDNA Labeling Kit and SuperScript™ Indirect cDNA Labeling System, 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.

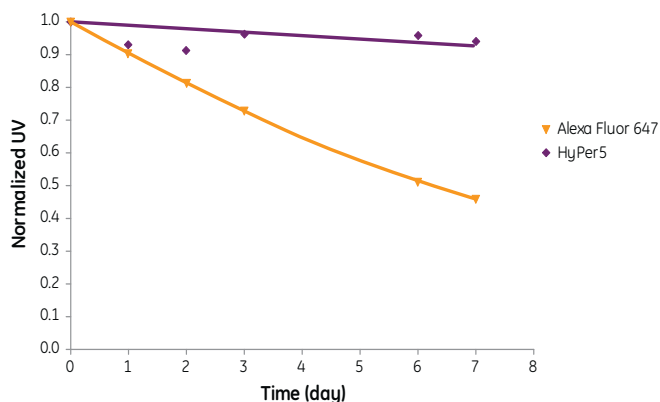


Fig 2. Comparative photostability of HyPer5 and Alexa Fluor 647. The free form of each dye was dissolved in 5 ml of water in a 20 ml borosilicate glass vial and adjusted to about 1 AU with water. Both dyes were 25 cm from an incandescent light source (11 W) and exposed for 7 d at 20°C and the absorbance for each was measured daily with a UV/vis spectrophotometer at 647 and 660 nm. Identical samples were placed in a light-proof container to obtain control data.

Ozone stability

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.

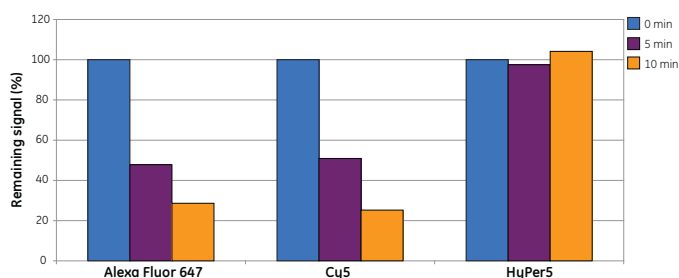


Fig 3. Ozone stability of HyPer5 dye showing the amount (%) of signal remaining from control genes after exposure of hybridized microarrays to ozone treatments at 100 ppb for 0, 5, and 10 min. Each hybridized microarray was rescanned for 4 min with a 2 min interval on a ScanArray at 633 nm. The data was derived from an average signal of 10 scorecard genes.

Labeling efficiency

HyPer5 NHS reactive dye

To determine the labeling efficiency of each dye, 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.

Table 2. Post-labeling data starting from 20 µg of total RNA with HyPer5, Cy5, and Alexa Fluor 647 NHS reactive dyes. The data shown represents the average of 35 HyPer5 and 23 Cy5 labeling reactions. The Alexa Fluor 647 data was produced with the SuperScript™ Indirect cDNA Labeling System (n = 3).

	HyPer5 NHS reactive dye	Cy5 NHS reactive dye	Alexa Fluor 647 NHS reactive dye
Dye incorporation (pmol)	87.1	34.5	12.4
Nucleotide per dye ratio	29	77	354

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.

	HyPer5 NHS reactive dye	Cy5 NHS reactive dye
Dye incorporation (pmol)	223	78
Nucleotide per dye ratio	14	42

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.

	HyPer5 NHS reactive dye	Cy5 NHS reactive dye
Nucleotide concentration (ng)	1229.41	752.94
Dye incorporation (pmol)	26.4	10.2
Nucleotide per dye ratio	47	74

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.

	HyPer5-dCTP	Cy5-dCTP
Dye incorporation (pmol)	44.7	43.2
Nucleotide per dye ratio	42	38

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.

	HyPer5-dCTP	Cy5-dCTP
Dye incorporation (pmoles)	32	35
Nucleotide per dye ratio	94	70

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.

Array	Cy5 or HyPer5 average signal	Cy3 average signal	Count of genes above background
HyPer5 array	2383	1847	4574
Cy5 array	2522	2292	4598

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.

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

Dye	Cy5 or HyPer5 average signal	Cy3 average signal	Count of genes above background
HyPer5 array	471	937	5458
Cy5 array	540	927	5858

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

1. DeRisi, J. L. *et al.* Exploring the metabolic and genetic control of gene expression on a genomic scale. *Science* **278**, 680-686 (1997).
2. Richter, A. *et al.* Comparison of Fluorescent Tag DNA Labeling Method Used for Expression Analysis by DNA Microarrays. *BioTechniques* **33** (3), 620-630 (2002).
3. Fare, T.L. *et al.* Effects of Atmospheric Ozone on Microarray Data Quality. *Anal Chem* **75**, 4672-4675 (2003).

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Ordering information

Product	Quantity	Code number
HyPer5 dCTP	25 nmol	28-9231-83
HyPer5 dCTP	250 nmol	28-9231-84
Multi pack containing 5 × 25 nmol Cy3 dCTP + 5 × 25 nmol HyPer5 dCTP		28-9231-85
Cy3 and HyPer5 Post-Labeling Reactive Dye Pack (12 × 40 000 pmol Cy3 + 12 × 15 000 pmol HyPer5)	1 pack	28-9224-19
HyPer5 Post-Labeling Reactive Dye Pack	12 × 15000 pmol	28-9224-20

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Amersham CyDye Fluorescent Nucleotides	
Amersham CyDye Value Packs (mono-Reactive NHS Ester)	
Amersham CyScribe First-Strand cDNA Labeling Kit	RPN6200
Amersham CyScribe Post-Labeling Kit	RPN5660
Amersham CyScribe Array CGH Genomic Labeling System	28-9199-56
illustra™ CyScribe GFX Purification Kit	27-9606-01
illustra GFX™ PCR DNA and Gel Band Purification Kit	28-9034-70
illustra GenomiPhi V2 DNA Amplification Kit	25-6600-30

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