

FAQs

FlashTag Biotin RNA Labeling Kit for Affymetrix GeneChip miRNA Arrays

How should I isolate/purify my RNA?

Any kit for purification of either total RNA or LMW RNA will be compatible with FlashTag. Ensure that the purification method retains low molecular weight species. Some commercial products that have been tested successfully with FlashTag include:

Marligen Vantage kits

Qiagen miRNeasy kits

Applied Biosystems miRVana kits

Should I enrich my total RNA for miRNA?

Using total RNA can save time and money, and prevent sample loss. However, some applications may require enrichment for optimal profiling. For example, to distinguish mature and precursor miRNAs, enrichment may be necessary. In addition, degraded total RNA samples should be enriched prior to FlashTag labeling.

Will total RNA labeling affect the miRNA profile on the array?

No, specificity is defined by the array itself, using the recommended hybridization conditions.

How much RNA is required for labeling?

Use either 0.1-3ug total RNA or the LMW RNA enriched from 0.1-3ug total RNA. For new users, 1ug of total RNA is recommended as a starting point for labeling.

How is PAP tailing of plant miRNA achieved?

The FlashTag kit contains a unique buffer (Vial 1) which enables PAP tailing of plant miRNA (and all RNA molecules).

What is Vial 5, FlashTag Ligation Mix?

Vial 5 contains Genisphere's proprietary 3DNA dendrimer, a branched structure of single and double stranded DNA conjugated with 15 biotins. The 3DNA dendrimer is attached to a poly(T) sequence that facilitates ligation to all poly(A) tailed RNA molecules.

What is Vial 8, RNA Spike Control Oligos?

Vial 8 consists of five oligos which are spiked into the RNA sample prior to FlashTag labeling. These oligos contain controls for the GeneChip miRNA array and the ELOSA QC Assay.

- Oligos 2, 23, and 29 are RNA, and confirm poly(A) tailing and ligation.
- Oligo 31 is poly(A) RNA, and confirms ligation.
- Oligo 36 is poly(dA) DNA, and confirms ligation and lack of RNAses in the RNA sample.

The Affymetrix library file lists the following names for these probe sets:

- spike in-control-2 st
- spike in-control-23 st
- spike in-control-29 st
- spike in-control-31 st
- spike in-control-36 st

Each probe set should show >1000 units (signal-background)

I've labeled my samples but not I'm ready to hybridize the GeneChip miRNA arrays. What should I do?

Store the biotin-labeled RNA samples on ice for up to 6 hours, or at -20°C for up to two weeks.

Can I label my samples in advance and hybridize arrays later?

Yes, store at -20°C for up to two weeks.

Is there a way to check for successful labeling prior to array hybridization?

Successful biotin labeling is verified via a simple colorimetric ELOSA assay through the hybridization of the biotin-labeled RNA Spike Control Oligos (Vial 8) to complementary ELOSA Spotting Oligos (Vial 9) immobilized onto microtiter plate wells. Refer to Appendix A of the FlashTag product manual.

Is it essential to run the ELOSA QC Assay procedure every time I use FlashTag?

Yes, Genisphere recommends running the ELOSA for every labeled sample. The labeled sample may be stored at -20°C for up to two weeks prior to running the ELOSA.

Is there a recommended vendor for dextran sulfate?

The following three sources of dextran sulfate have been successfully tested on arrays:

- Add 5mL 50% dextran sulfate, **Millipore** cat. no. S4030, to 5mL nuclease-free water. Resuspend by vortexing.
- Bring 2.5g Dextran Sulfate, **Sigma** cat. no. D8906, to 10mL with nuclease-free water. Resuspend by vortexing.
- Bring 2.5g Dextran Sulfate, **USB** cat. no. 70796, to 10mL with water. Heat at 55°C for up to 5 hours and vortex periodically until resuspended.

Once prepared, store 25% dextran sulfate at room temperature, up to 4 weeks.

Only **Millipore** Dextran Sulfate has been successfully tested in the ELOSA QC Assay.

Are there any other concerns for running FlashTag?

All materials (tubes, tips, etc.) should be nuclease-free, and all reagents should be prepared with nuclease-free components.

Can I re-use my hybridization cocktail?

Yes, and if necessary, add more volume of 1X hybridization mix prior to re-hybridization. Refer to Appendix B, Array Rehybridization procedure.

What is the difference between KR8870 and FT10AFYB/FT30AFYB?

The KR8870 kit does not contain controls that are required for Affymetrix GeneChip miRNA arrays. The only kits recommended for GeneChip miRNA arrays are FT10AFYB and FT30AFYB.

How do I contact Genisphere Technical Support?

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