

# User Guide - ULS Labeling of microRNA



<b>Version 1.0</b>	(February 2009)
<b>Product:</b>	ULS™ microRNA Labeling Kit
<b>Catalog number:</b>	EA-036, EA-037 and EA-038
<b>Lot number:</b>	See label on package
<b>Unit Size:</b>	10 dual color labeling reactions or 20 single color labeling reactions
<b>Storage conditions:</b>	See below

## **IMPORTANT NOTE:**

This user guide contains minimal information to provide experienced users with a short protocol to use at the bench! A more detailed, full manual can be downloaded from our website: <http://www.kreatech.com>

## **I. RNA quality**

For total RNA isolation we recommend to use Trizol (Invitrogen) extraction followed by precipitation or microRNA enrichment using any of the following kits: miRNeasy™ procedure (QIAGEN), *mirVana*™ miRNA Isolation procedure (Ambion/Applied Biosystems), miRACLE™ miRNA isolation kit (Stratagene).

Regardless what source of RNA is being used, the following quality criteria should be met:

- For all RNAs  $OD_{260}/OD_{280}$  should be  $>1.90$
- $OD_{260}/OD_{230}$  should be  $>2.10$

**Be aware that some components in silica based purification systems inhibit the ULS labeling reaction. This can be prevented by a final wash step using 80% ethanol (PA) before elution, followed by elution using ultrapure water instead of elution buffer**

## **II. ULS Labeling of total RNA or microRNA enriched RNA**

Note: the procedure described below is assuming the need to hybridize 1 µg of total RNA or microRNA enriched RNA per sample on a microarray. In case your application needs more or less sample to be hybridized, you can scale up or down accordingly: use a ratio of exactly 1.0 µL of ULS per 1.0 µg of RNA. The optimal RNA concentration for ULS labeling is  $\geq 50$  ng/µL. If less than 1 µg RNA is available, use lower labeling volumes in order to aim for a RNA concentration of 50 ng/µL (e.g. if only 250 ng RNA is available the optimal labeling volume is 5 µL)

1. Briefly spin all required reagents to drive the contents off the walls and lid.
2. Pre-heat a waterbath OR thermocycler at 85°C.
3. Add RNA and ultrapure water to the tube to a total volume of 17.0 µL
4. Add 2.0 µl of 10 x Labeling Solution to obtain a 1 x Labeling Solution.
5. Add 1.0 µl of Cy3-ULS or Cy5-ULS.
6. Incubate sample for 15 minutes at 85°C.
7. Place samples directly on ice and spin down to drive the contents off the walls and lid.
8. Continue with the KREApure purification to remove non- reacted ULS dyes (section III).

Example of Cy-Uls labeling of 1 µg of total RNA or microRNA enriched RNA

	Labeling mixture
RNA	x µL (= 1.0 µg)
Cy-Uls	1.0 µL
10 x labeling solution	2.0 µL
Ultrapure water	17 - x µL
<b>Total volume</b>	<b>20 µL</b>

**NOTE:** Be aware that the ratio µL ULS compound vs. µg RNA is always exactly 1 : 1.

### III. Removal of non-reacted ULS dyes using KREApure columns

- Optional: If lower labeling volumes are used adjust volume to 20 µL using ultrapure water.
1. Resuspend column material by vortexing
  2. Loosen cap ¼ turn and snap off the bottom closure
  3. Place the column in a 2 mL collection tube
  4. Pre-spin the column for 1 minute at 20,800 x g (i.e. maximum speed of a typical table-top microcentrifuge)
  5. Discard flow-through and column cap, but re-use collection tube
  6. Add 300 µL ultrapure water to the column and centrifuge for 1 min at 20,800 x g
  7. Discard collection tube and flow through
  8. Place column in a nuclease-free 1.5 mL microcentrifuge tube (not provided)
  9. Add ULS-labeled RNA onto the center of the column bed.
  10. Centrifuge column for 1 minute at max speed using a tabletop microcentrifuge.
  11. Flow-through contains the purified, labeled RNA.
  12. Determine the degree of labeling (DoL) by measuring absorbance at 260nm and 550nm (for Cy3-Uls) or 650nm (for Cy5-Uls)
  13. Calculate the Density of Labeling (DoL) value using the interactive calculator on our website ([www.kreatech.com](http://www.kreatech.com))
  14. Store the sample (- 20°C) or proceed with the hybridization procedure.

### IV. Preparation of labeled total RNA using KREAblock solution (optional) for hybridization

1. For dual color assays, pool the labeled samples.
2. Concentrate (using a concentrator) to nearly dryness.
3. After concentration, dissolve the labeled material in ¼ volume of ultrapure water and add ¼ volume KREAblock (optional; otherwise add another ¼ volume of ultrapure water).
4. Add ½ volume of 2 x Hybridization buffer.

This product is intended for RESEARCH USE ONLY. IT IS NOT INTENDED FOR DIAGNOSTIC APPLICATIONS and/or COMMERCIAL PURPOSES.

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