User Guide - ULS Labeling of microRNA

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/ - this user guide is meant for the ULS microRNA Labeling Kits (product EA-036/037/038).

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), *mir*Vana™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 ≥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 KREA*pure* 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 miixture
RNA	x µL (= 1.0 µg)
Cy - ULS	1.0 µL
10 x labeling solution	2.0 µL
Ultrapure water	17 - x µL
Total volume	**20 µL**

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 KREA*pure* 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)
14. Store the sample (- 20℃) or proceed with the hybridization procedure.

IV. Preparation of labeled total RNA using KREA*block* solution (optional) forhybridization

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