Quick Protocol

Version 1.0 (Feb 2007)

Product: **KREA*pure*™ columns**
Description: **Dye removal purification column**
Catalog number: KP-020, KP-050
Lot number: See label on the outside of package
Unit Size: KP-020 (20 pcs), KP-050 (50 pcs)
Storage conditions: KREA*pure*™ columns are stable at 4°C. Do not freeze. See label for expiry date.

Introduction:

KREA*pure*™ columns are specifically designed to remove unreacted ULS™ from your reaction mixture (see Fig.1 for overview). Free ULS™ is removed with a 99.9% efficiency. Since KREA*pure*™ specifically targets free ULS™ and has no affinity for nucleic acids, both RNA and DNA have a high recovery over these columns. A 95% recovery is standard (Fig.2). The KREA*pure*™ column is not size discriminant and therefore fragments of all sizes pass through the column unhindered making it ideal for all applications where small fragments are of interest e.g. miRNA studies.

Application:

KREA*pure*™ columns can be used in collaboration with other ULS™ based kits from Kreatech such as Platinum*Bright*™ (e.g. GLK-001), ULS™ arraCGH Kits (e.g. EA-005) and ULS™ aRNA Fluorescent Labeling Kits (e.g. EA-006). **The KREA*pure*™ columns are specific for ULS™ only.**

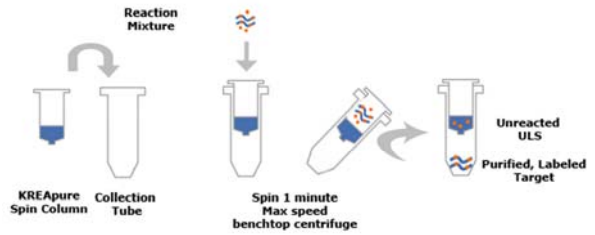

Figure 1: Overview of KREA*pure*™ Purification Procedure. KREA*pure*™ columns are specifically designed to remove non-reacted ULS™ from your reaction mixture. Since KREA*pure*™ specifically targets free ULS™ and has no affinity for nucleic acids, both RNA and DNA have a high recovery over these columns.

Protocol:

N.B. An important factor to consider before beginning the dye removal is that the KREA*pure*™ column capacity is 50 µL. If the labeling reaction volume exceeds this then concentration will be necessary before purification over the KREA*pure*™ column can be carried out. Please refer closely to the specific manual that came with your original labeling kit for sample preparation and labeling.
(See also www.kreatech.com)

Removal of free ULS™ label using KREApure columns

(20800 x g is equivalent to 14,000 rpm on eppendorf 5417C)

1. Resuspend column material by vortexing
2. Loosen cap 1/4 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 20800 x g
5. Discard flow-through and re-use collection tube
6. Add 300 µL DNase free water to the column
7. Spin column for 1 minute at 20800 x g
8. Discard collection tube and flow-through
9. Put the column in a new (DNase free) 1.5 mL micro centrifuge tube
10. Add ULS-labeled nucleic acid on to column bed – be careful not to pipette to the sides of the column but directly onto the column material
11. Spin the column for 1 minute at 20800 x g
12. The flow through is purified labeled material
13. If required the degree of labeling (DOL) can be measured at this point

Your sample is now labeled, purified and ready for downstream applications.

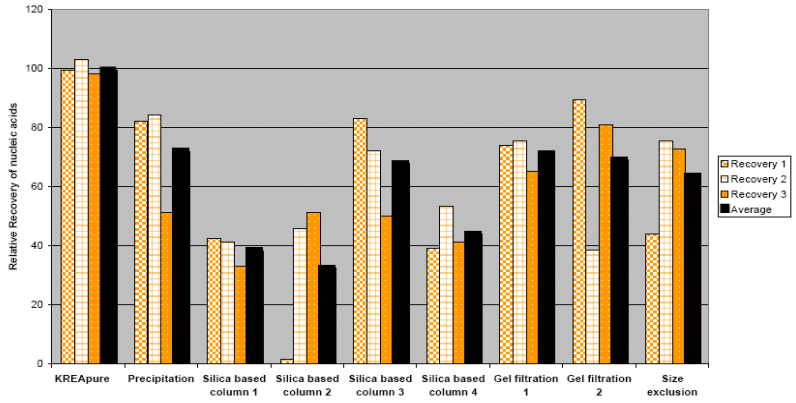

Figure 2: Relative recovery of labeled nucleic acids using KREA*pure*™ column and other purification procedures. This figure shows the nucleic acid recoveries of triplicate purifications using 9 methods. The result is that KREA*pure*™ purification gives a significantly higher recovery of nucleic acids than recovery of nucleic acids than other commonly used methods and is very reproducible

Lab Notes:

For further information on KREA*pure*™ columns and also the ULS™ technology please go to www.kreatech.com

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