PAGExt: PAGE extraction kit

Product	Catalog no	Rxns
PAGExt	#KGE-002	18

Shipping: 4°C

Storage Conditions: store components according to this manual

Shelf Life: 12 months

Description: IMMAGINA PAGE extraction kit is designed for rapid and efficient PAGE extraction of RNA Fragments and DNA Libraries.

Version 032021

List of components

Product (label)	Catalog no	Store Conditions	Quantity
Filters tubes bag (18 pieces)	#KGE002-6	RT	1
Pierced tubes bag (18 pieces)	#KGE002-7	RT	1
TR buffer (TR)	#KGE002-1	4°C	0.5 mL
RNA Extraction Buffer (REB)	#KGE002-2	4°C	8 mL
DNA Extraction Buffer (DEB)	#KGE002-3	4°C	8 mL
Marker 1 (M1)	#KGE002-4	-20°C	10 µL
Marker 2 (M2)	#KGE002-5	-20°C	10 µL

Shelf life: 12 months from the delivery date.

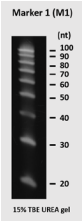
Additional Materials Required:

- 15% TBE-Urea polyacrylamide gel (e.g. BioRad catalog no. 450-6053 or Thermo Scientific catalog no. EC6885BOX)
- 10% TBE polyacrylamide gel (e.g. Thermo Scientific catalog no. EC6275BOX)
- Gel Loading Buffer II (Denaturing PAGE) (Thermo Scientific catalog no. AM8546G)
- DNA Gel Loading Dye (e.g. Thermo Scientific catalog no. R0611)
- SYBR Gold (Thermo Scientific, catalog no. S11494)
- GlycoBlue (Ambion catalog no. AM9515)
- Isopropanol (Sigma catalog no. 278475)
- Microcentrifuge and nonstick RNase-free microfuge tubes (0.2 mL and 1.5 mL)

Work always in an RNase-free environment.

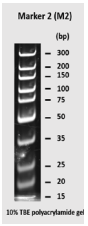
RNA fragments PAGE Purification

Pre-run the gel at 200 V for 30 min in TBE prepared with nuclease-free water. ***Clean well the gel wells with a syringe to remove UREA residuals before to load the samples.***



- Prepare samples: add 5 µL of Gel Loading Buffer II to 5 µL of purified RNA fragments;
- Prepare M1 marker: mix 1 µL M1, 4 µL nuclease-free water and 5 µL of Gel Loading Buffer II;
- Denature the samples and marker M1 for 90 s at 80 °C. Place the tubes immediately on ice;
- Load the samples and marker on 15% TBE-urea polyacrylamide gel and run the gel at 200 V until the bromophenol blue band reaches the bottom of the gel;
- Stain the gel with SYBR Gold and visualize the RNA using a Transilluminator;
- Size select the RNA fragments according the marker M1.
- Place each gel slice in a pierced tube (provided) and nest the pierced small tube inside a 1.5-ml RNase-free Microfuge tube (provided). Spin at maximum speed for 3 min at 4°C. Transfer carefully any remaining gel debris from the pierced tube and discard the pierced tube;
- Add 400 µL of REB (RNA Extraction Buffer), close the vial with the provided cup, incubate the tubes for 1 hour at - 80°C, thaw them at RT and then place the samples on a wheel in slow motion (3 rpm), at RT overnight;
- With a 1 mL cut-tip, transfer the liquid and gel slurry into a spin filter (provided) and spin at 650g for 3 min at 4°C to remove the gel debris. Transfer the eluted solution to a new 1.5 ml tube (not provided);
- Add 700 µL of isopropanol and 1.5 µL GlycoBlue to the eluted sample;
- Store at - 80°C for 2h (fast procedure) or overnight (safe procedure);
- Thaw the samples on ice and pellet the RNA by centrifugation (20000g) for 30 min at 4°C and air-dry the pellet;
- Resuspend the pellet in 11 µL TR buffer.
- Proceed with RNA fragments quantification.

Library PAGE Purification



- Prepare samples: add 4 µL of 6x DNA loading dye II to 20 µL of cleaned up PCR;
- Prepare M2 marker: mix 1 µL M2, 9 µL nuclease-free water and 2 µL of 6x DNA loading dye;
- Load the samples and marker on 6-10% TBE polyacrylamide gel (split the sample total volume into 2 adjacent lanes) and run the gel at 200 V until the bromophenol blue band reaches the bottom of the gel;
- Size select the library according to M2;
- Place each gel slice in a pierced tube (provided) and nest the pierced small tube inside a 1.5-ml RNase-free Microfuge tube (provided). Spin at maximum speed for 3 min at 4°C. Transfer carefully any remaining gel debris from the pierced tube and discard the pierced tube;
- Add 400 µL of DEB (DNA Extraction Buffer), close the vial with the provided cup, incubate the tubes for 1 hour at - 80°C, thaw them at RT and then place the samples on a wheel in slow motion (3 rpm), at RT overnight;
- With a 1 mL cut-tip, transfer the liquid and gel slurry into a spin filter (provided) and spin at 650g for 3 min at 4°C to remove the gel debris. Transfer the eluted solution to a new 1.5 ml tube (not provided);
- Add 700 µL of isopropanol and 1.5 µL GlycoBlue to the eluted sample;
- Store at - 80°C for 2h (fast procedure) or overnight (safe procedure);
- Thaw the samples on ice, pellet the library by centrifugation (20000g) for 30 min at 4°C and air-dry the pellet;
- Resuspend the pellet in 11-15 µL TR buffer
- Proceed with Library validation and quantification.

Related products

Product	Catalog no	Rxns.
RiboLace Ribo-Seq - Module 1	#RL001_mod1	9
LACESeq	#LS-001	9
LACESeq iUDI plates	#UDI-LS-001	32

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Contacts

Info
info@immaginabiotech.com

Sale support (quoting, ordering and order status update)
orders@immaginabiotech.com

Technical service (technical enquiries and quality complaints)
techsupport@immaginabiotech.com

Via Sommarive, 18, 38123,
TRENTO, ITALY

www.immaginabiotech.com

+39 0461312018

Notes: