



## PAGExt: PAGE extraction kit

Product	Catalog no	Rxns.
PAGExt	#KGE002_12	24 (12 RNA + 12 DNA gel extraction)

Shipping: Blue Ice and Dry ice

Storage Conditions: store components according to storage conditions reported on labels and on 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.

Suitable for: Eukaryotic cell lines and tissues

*For Research Use Only. Not Intended for Diagnostic or Therapeutic Use.*




<b>Kit contents</b>	<b>Qty.</b>	<b>Storage</b>
PAGExt +4°C components	1 box	4°C
PAGExt -20°C components	1 Alu-bag	-20°C
Filters and Tubes	1 package	RT

### **Additionally Required Materials**

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

# PAGExt extraction kit


PAGExt extraction kit components needed in this part:

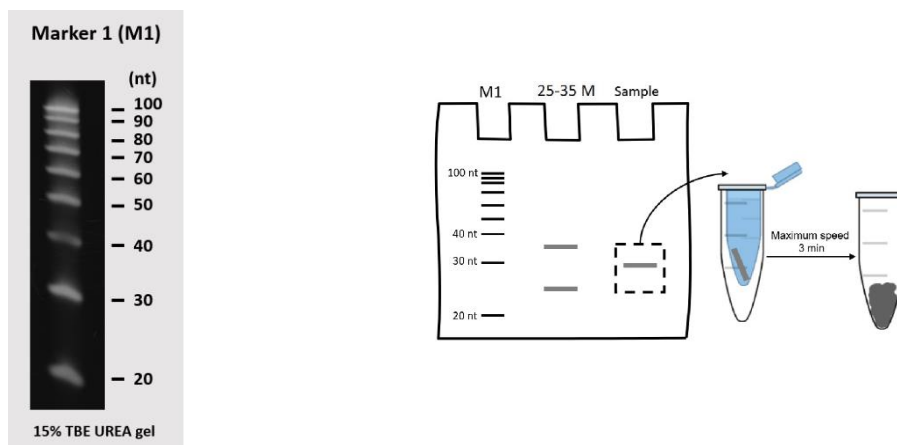
Kit component	Cat. nr.	Quantity	Storage	Type	Vial
Filters tubes	#KGE002-6	24 pcs	RT	Bag	--
Pierced tubes	#KGE002-7	24 pcs	RT	Bag	--
TR buffer (TR)	#KGE002-1	0.5 mL	4°C	Vial	 yellow
RNA Extraction Buffer (REB)	#KGE002-2	5.2 mL	4°C	Bottle	--
DNA Extraction Buffer (DEB)	#KGE002-3	5.2 mL	4°C	Bottle	--
Marker 1 (M1)	#KGE002-4	13 µL	-20°C	Vial	 yellow
Marker 2 (M2)	#KGE002-5	13 µL	-20°C	Vial	 yellow

## 1. PAGE PURIFICATION OF THE RIBOSOME PROTECTED FRAGMENTS

**⚠ 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 loading the samples.**

- ☐ **1.1 Prepare samples:** add 5 µL of Gel Loading Buffer II to 5 µL of RPFs obtained from Step 5.
- ☐ **1.2 Prepare M1 marker:** mix 1 µL M1, 4 µL nuclease-free water and 5 µL of Gel Loading Buffer II.
- ☐ **1.3 Prepare 25-35 Marker:** mix 2 µL of 25-35 Marker, 3 µL nuclease-free water and 5 µL Gel Loading Buffer II.
- ☐ **1.4 Denature the samples, 25-35 Marker and marker M1** for 90 s at 80 °C. Place the tubes immediately on ice.
- ☐ **1.5 Load the samples, 25-35 Marker, and Marker 1** on 15% TBE-urea polyacrylamide gel and run the gel at 200V until the bromophenol blue band reaches the bottom of the gel.
- ☐ **1.6 Stain the gel with SYBR Gold and visualize the RNA** using a UV-Transilluminator.
- ☐ **1.7 Size select the ribosome protected fragments (RPF)** between 25-nt and 35-nt according to the marker M1 and 25-35 Marker (see Figure below).

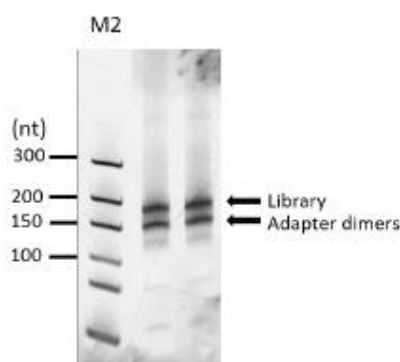
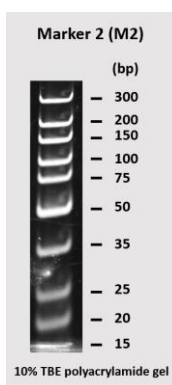
 The RPFs are ~25-35 nts in length. The 25-35 Marker, is a mix of two oligos 25 nt and 35 nt in length. It can be used as a size marker.



- ☐ **1.8** Place each gel slice in a pierced tube (provided) and nest the pierced small tube inside a 1.5-ml RNase-free Microcentrifuge tube (provided). Spin at maximum speed for 3 min at 4°C. Transfer carefully any remaining gel debris from the pierced tube, discard the pierced tube and keep the 1.5 ml microcentrifuge tube.
- ☐ **1.9** Add 400 µL of RNA Extraction Buffer (REB), 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.
- ☐ **1.10** With a 1 mL cut-tip, add the gel slurry to the provided filter tube and spin at 650g for 3 min at 4°C to remove the gel debris. Transfer the eluted solution to a new tube.
- ☐ **1.11** Add 700 µL of isopropanol and 1.5 µL GlycoBlue to the eluted sample.
- ☐ **1.12** Store at - 80°C for 2h (fast procedure) or overnight (safe procedure).
- ☐ **1.13** Thaw the samples on ice and pellet the RNA by centrifugation (20000g) for 30 min at 4°C.
- ☐ **1.14** Remove the supernant and wash the pellet once with 70% cold ethanol. Centrifuge for 5 min at 20000g, 4°C.
- ☐ **1.15** Remove the supernant and resuspend the pellet in 11 µL TR buffer.
- ☐ **1.16** Quantify the RPFs (1 µL) using a Qubit™ microRNA Assay Kit.

## 2. PAGE PURIFICATION OF LIBRARIES

- **2.1 Prepare samples:** add 4  $\mu$ L of 6x DNA loading dye to 20  $\mu$ L of cleaned-up PCR (from Step 12.3);
- **2.2 Prepare M2 marker:** mix 1  $\mu$ L M2, 9  $\mu$ L nuclease-free water and 2  $\mu$ L of 6xDNA loading dye;
- **2.3 Load the samples and marker on a 10% TBE polyacrylamide gel** (split the sample total volume into 2 adjacent lanes) and run the gel for 50 min at 200V. If the loading dye contains xylene cyanol, run the gel till the xylene cyanol reaches the bottom of the gel.
- **2.4 Stain the gel with SYBR Gold and visualize the libraries using a UV-Transilluminator.**
- **2.5 Excise the library band at ~ 200 nt according to M2 (see Fig. below);** take care not to excise the ~170 nt adapter dimers band!
- **2.6 Place gel slice in a pierced tube (provided) and nest the pierced small tube inside a 1.5-ml RNase-free Microcentrifuge tube (provided).** Spin at maximum speed for 3 min at 4°C. Transfer carefully any remaining gel debris from the pierced tube, discard the pierced tube and keep the 1.5 ml microcentrifuge tube.
- **2.7 Add 400  $\mu$ L 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 at RT overnight.**



- **2.8 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;
- **2.9 Add 700  $\mu$ L of Isopropanol and 1.5  $\mu$ L GlycoBlue to the eluted sample;**
- **2.10 Store at - 80°C for 2h (fast procedure) or overnight (safe procedure).**
- **2.11 Thaw the samples on ice and pellet the RNA by centrifugation (20000g) for 30 min at 4°C.**
- **2.12 Remove the supernant and wash the pellet once with 70% cold ethanol. Centrifuge for 5 min at 20000g, 4°C.**
- **2.13 Remove the supernant and resuspend the pellet in 11-15  $\mu$ L TR buffer. Proceed with Library Quality check and quantification.**

## Contacts



### Info

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