PAGExt

PAGE extraction kit for both RPF and library size selection

Product	Catalog no	Rxns.
PAGExt	KGE00-12	24

Shipping: Blue Ice and Dry ice

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.

Suitable for: Eukaryotic cell lines and tissues

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

Table of Contents

Kit storage info

	Qty.	Storage
4°C components	1 box	4°C
-20°C components	1 box	-20°C
Filters and Tubes	1 bag	RT

Table 1. *Kit composition (in boxes and bag) and storage temperature.*

Additionally Required Materials

- RNAse-free water
- Microcentrifuge and non-stick RNase-free microfuge tubes (0.2 mL and 1.5 mL)
- Automatic wheel (rotator)
- GlycoBlue (Ambion catalog no. AM9515)
- Isopropanol (Sigma catalog no. 278475)
- 15% TBE-Urea polyacrylamide gel (e.g. BioRad catalog no. 450-6053 or Thermo Scientific catalog no. EC6885BOX)
- Gel Loading Buffer II (Denaturing PAGE) (Thermo Scientific catalog no. AM8546G)
- Ultra-low range molecular weight marker (i.e., Thermo Scientific catalog no. 10597012 or similar)
- SYBR Gold (Thermo Scientific, catalog no. S11494)
- Qubit Fluorometer
- Qubit™ microRNA Assay Kit
- Agilent 2100 Bioanalyzer
- Agilent High Sensitivity DNA Kit (Agilent Tech. catalog no. 5067-4626)
- 10% TBE polyacrylamide gel (e.g. Thermo Scientific catalog no. EC6275BOX)
- DNA Gel Loading Dye (e.g., Thermo Scientific catalog no. R0611)

Step B6. RPF EXTRACTION: GEL RUN

PAGExt kit components and additional required materials needed in this section:

Step N	Kit component	Cat. nr.	Volume	Storage	Type	Vial cap color
B6	15% TBE-Urea polyacrylamide gel	Additionally Required Material				
B6	Gel Loading Buffer II	Additionally Required Material				
B6	25-35 Marker (25-35 M)	# IBT0131	15 µL	-80°C	Vial	clear
B6	Marker 1 (M1)	# IBT0401	13 µL	-80°C	Vial	Yellow
B6	SYBR Gold	Additionally Required Material				
B7	Filter tubes	# IBT0391	24 pcs	RT	Bag	
B7	Pierced tubes	# IBT0371	24 pcs	RT	bag	
B7	RNA extraction buffer (REB)	# IBT0381	15 mL	4°C	Bottle	
B7	TR buffer	#IBT0351	500 µL	4°C	Vial	Yellow
B7	Isopropanol	Additionally Required Material				
B7	GlycoBlue	Additionally Required Material				
B7	Qubit™ microRNA Assay Kit	Additionally Required Material				

NOTE: RNA extraction buffer (REB) contains SDS, thus a cloudy solution might appear when stored at 4°C. If cloudy, before using the solution, warm it at RT and mix it until the solution is clear.

The RNA recovered after RiboLace pulldown contains the ribosome-protected fragments (RPFs) that need to be purified. 1 µL of this RNA can be quantified by Nanodrop and at least 1.5 µg of RNA should be extracted. All the extracted RNA should be run on gel, depending on the retrieved quantity, it should be run in one or multiple lanes. Please run up to 2 µg of extracted RNA in one single lane. E.g., if 3 µg are extracted after pulldown, please divide the sample in two lanes. In case the RPFs are not visible on the gel, please contact our tech support (techsupport@immaginabiotech.com).

- ☐ **B6.1** 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.
- ☐ **B6.2** Prepare samples: add 10 µL of Gel Loading Buffer II to 10 µL of RNA (1:1 volume).
- ☐ **B6.3** Prepare 25-35 Marker: mix 2 µL of 25-35 Marker, 3 µL nuclease-free water, and 5 µL Gel Loading Buffer II. And prepare Marker 1: mix 1 µL of Marker 1, 4 µL nuclease-free water, and 5 µL Gel Loading Buffer II.
- ☐ **B6.4** Use also an ultra-low range molecular weight marker as a reference.
- ☐ **B6.5** Load the samples, the 25-35 Marker and the 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 (about 50 min to 1 hour).
- ☐ **B6.6** Stain the gel for 5 minutes with SYBR Gold in TBE and visualize the RNA using a UV-Transilluminator and proceed with the PAGE gel extraction.

Step B7. PAGE EXTRACTION OF THE RPF

☐ **B7.1** After gel visualization, please note that a signal between 25 nt and 35 nt should be visible. Bands present in this area belong to the 80S ribosome-protected fragments (RPFs) and their presence in the gel indicates a proper ribosome pulldown (Figure 1).

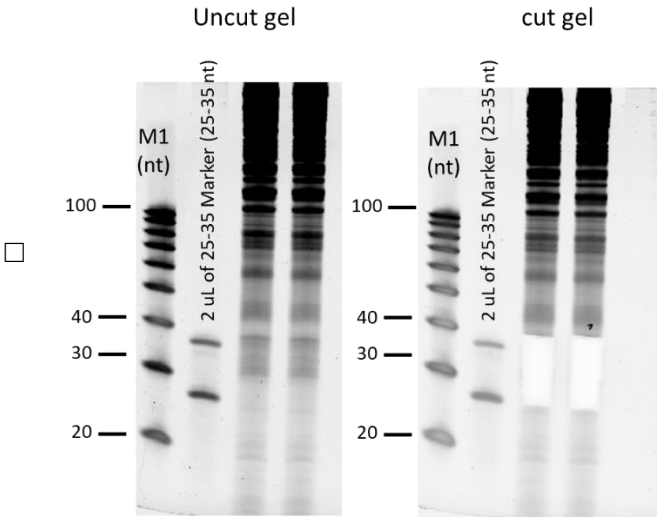

Fig.1 The RPFs are ~25-35 nt in length. The 25-35 Marker is a mix of two oligos 25 nt and 35 nt in length. It can be used as a size marker. Please note that the part that needs to be cut is between 25-35 depicted in the white rectangle on right gel.

☐ **B7.2** RPFs need to be extracted from gel by size selection of the RPF between 25-nt and 35-nt according to the marker M1 and 25-35 Marker (white rectangle removed from Fig.1 right).

☐ **B7.3** 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 RT. If part of the gel pieces is still in the pierced tube, repeat the spinning. In any case, transfer carefully any remaining gel debris from the pierced tube, discard the pierced tube and keep the 1.5 ml microcentrifuge tube.

☐ **B7.4** Add 400 µL of **RNA Extraction Buffer** (**REB**), close the vial with the provided cap and incubate the tubes for 1 hour at - 80°C. Thaw the tubes at RT and then place the samples on a wheel in slow motion (3-10 rpm), at RT overnight.

☐ **B7.5** With a 1 mL cut-tip, add the gel slurry to the provided filter tube and spin at 1,000g for 3 min at RT to remove the gel debris. Transfer the eluted solution to a new tube.

☐ **B7.6** Add 700 µL of isopropanol and 1.5 µL GlycoBlue to the eluted sample.

☐ **B7.7** Store at - 80°C for 2h (fast procedure) or overnight (safe procedure).

☐ **B7.8** Thaw the samples on ice and pellet the RNA by centrifugation at 20,000g for 30 min at 4°C.

☐ **B7.9** Remove the supernatant and wash the pellet once with 500 µL of 70% cold ethanol. Centrifuge for 5 min at 20,000g, 4°C.

☐ **B7.10** Remove the supernatant being careful to get rid of all ethanol residues. Resuspend the pellet in 12 µL TR buffer.

☐ **B7.11** Quantify the RPFs (2 µL) using a Qubit™ microRNA Assay Kit.

Step C7. LIBRARY EXTRACTION: GEL RUN

PAGExt kit components and additional required materials needed in this section:

Step N	Kit component	Cat. nr.	Volume	Storage	Type	Vial cap color
C7	10% TBE polyacrylamide gel	Additionally Required Material				
C7	DNA loading dye	Additionally Required Material				
C7	Marker 2 (M2)	# IBT0421	13 µL	-80°C	Vial	Yellow
C7	SYBR Gold	Additionally Required Material				
C8	Filter tubes	# IBT0391	24 pcs	RT	Bag	
C8	Pierced tubes	# IBT0371	24 pcs	RT	bag	
C8	DNA extraction buffer (DEB)	# IBT0411	15 mL	4°C	Bottle	
C8	TR buffer	#IBT0351	500 µL	4°C	Vial	Yellow
C8	Isopropanol	Additionally Required Material				
C8	GlycoBlue	Additionally Required Material				

☐ **C7.1** Prepare samples: add 4 µL of 6x DNA loading dye to 20 µL of cleaned-up PCR (from Step 12.3).

☐ **C7.2** Prepare M2 marker: mix 1 µL M2, 9 µL nuclease-free water and 2 µL of 6xDNA loading dye.

☐ **C7.3** Load the samples in two separate lanes, while Marker 2 in one lane on 10% TBE polyacrylamide gel. 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.

☐ **C7.4** Stain the gel for 5 minutes with a solution made of 10 mL of TBE and 1.5 µL of SYBR Gold and visualize the RNA using a UV-Transilluminator.

Step C8. PAGE EXTRACTION OF LIBRARY

☐ **C8.1** Excise the library band at ~ 200- nt according to M2 (see Figure 2); take care not to excise the ~170 nt adapter dimers band!

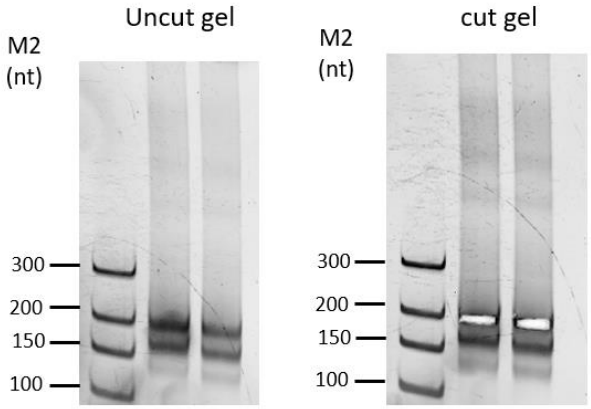

Fig.2 Example of library run on 10% TBE gel. The library is 200 nt length, while the adaptor dimer is 170 nt. Please note that the part that needs to be cut is exactly the band at 200 nt, leaving the whiskies out as depicted in the white rectangle on right gel. The sharper the cut, the lower the quantity of adaptor dimer that remains in the final library.

☐ **C8.2** 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 RT. If part of the gel pieces is still in the pierced tube, repeat the spinning. In any case, transfer carefully any remaining gel debris from the pierced tube, discard the pierced tube and keep the 1.5 ml microcentrifuge tube.

☐ **C8.3** Add 400 µL of **DEB** (**DNA Extraction Buffer**), close the vial with the provided cap, incubate the tubes for 1 hour at -80°C. Thaw the tubes at RT and then place the samples on a wheel in slow motion (3-10 rpm), at RT overnight.

☐ **C8.4** With a 1 mL cut-tip, transfer the liquid and gel slurry into a spin filter (provided) and spin at 1,000g for 3 min at RT to remove the gel debris. Transfer the eluted solution to a new 1.5 ml tube.

☐ **C8.5** Add 700 µL of **Isopropanol** and 1.5 µL **GlycoBlue** to the eluted sample.

☐ **C8.6** Store at - 80°C for 2h (fast procedure) or overnight (safe procedure).

☐ **C8.7** Thaw the samples on ice and pellet the DNA by centrifugation (20,000g) for 30 min at 4°C.

☐ **C8.8** Remove the supernatant and wash the pellet once with 500 µL of 70% cold ethanol. Centrifuge for 5 min at 20,000g, 4°C.

☐ **C8.9** Remove the supernatant and resuspend the pellet in 11 µL TR buffer. Proceed with Library Quality Check

Step C9. LIBRARY QUALITY CHECK

☐ **C9.1** Evaluate each size selected library by **Agilent 2100 Bioanalyzer** using the **Agilent High Sensitivity DNA Kit**.

☐ **C9.2** Use the library profile results to determine whether each sample is suitable for sequencing. Successful library production should yield a major peak at ~200-220 bp (see Fig. 5). Additional peaks might be observed at about 170-190 bp that originate from adapter dimers. If the peak areas are higher than 50% of the principal 200 bp peak, you need to purify again the libraries from gel before proceeding with sequencing.

☐ **C9.3** Perform a qPCR analysis using **P5 and P7 primers** on each library for highly accurate library quantification.

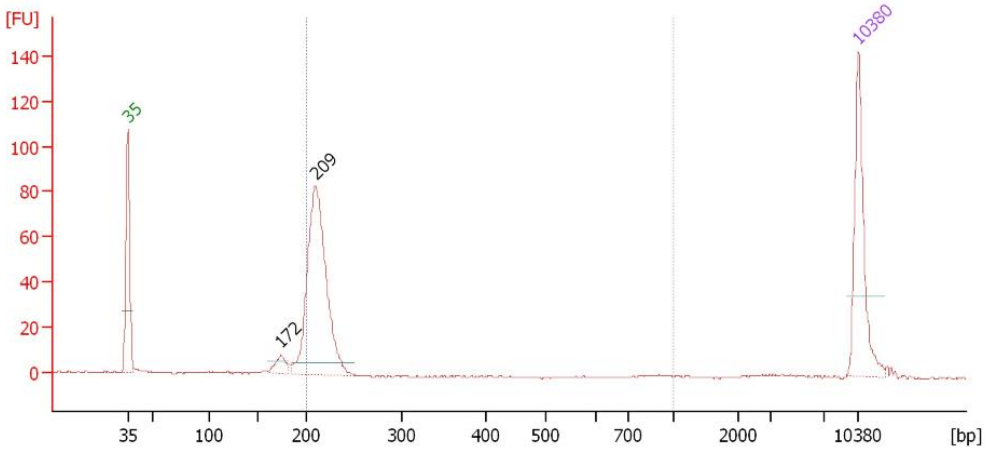

Figure 2. Example electropherogram libraries results. Typical electropherogram for a extracted library prepared with an immortalized cell line. The library was analyzed on an Agilent 2100 Bioanalyzer using the Agilent High Sensitivity DNA Kit. The electropherogram need to present at least one major peak between 200 to 220. In this example, the peak at 209 bp corresponds to the size of RPFs, while the peaks at 172 bp correspond with the size of adaptor dimers**.**

Contacts

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Notes: