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QUANT™ SEQ
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Globin Block Modules for QuantSeq
User Guide

Catalog Numbers:
070 (RS-Globin Block, *Homo sapiens*, 96 rxn)
071 (RS-Globin Block, *Sus scrofa*, 96 rxn)

070UG365V0101

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1. Overview

This User Guide outlines the protocol for using the Globin Block Modules for QuantSeq, with the QuantSeq 3′ mRNA-Seq Library Prep Kits for Illumina (FWD, Cat. No. 015, 113 - 115, 129 - 131 and REV, Cat. No. 016). QuantSeq uses total RNA as input with oligo(dT) priming to generate first strand cDNA. RNA removal is then performed and second strand synthesis is initiated by random priming. Final library amplification by PCR adds complete Illumina-compatible sequencing adapters and unique indices. For more detailed information about these protocols, please refer to the complete QuantSeq 3′ mRNA-Seq User Guide available for download at our website (www.lexogen.com/docs/quantseq).

Lexogen's Globin Block Modules block the generation of library fragments from the abundant and highly stable globin mRNAs that are present in whole blood. In mammals, the most abundant globin mRNAs are transcribed from the haemoglobin alpha and beta globin chain genes (*HBA1*, *HBA2*, and *HBB*). The Globin Block Modules consist of a modified RNA Removal Solution (RS-Globin Block), containing species-specific oligos complimentary to these highly abundant globin mRNAs.

The RS-Globin Block solutions (**RS-GB** ●) replace the standard RNA Removal Solution (**RS** ○) at the RNA removal step of the standard QuantSeq 3′ mRNA-Seq Library Prep protocol. The oligos bind to the first strand cDNA and prevent the generation of amplifiable library fragments from globin mRNAs during second strand synthesis.

Globin Block Modules are available for human (RS-Globin Block, *Homo sapiens,* Cat. No. 070.96) and pig (RS-Globin Block, *Sus scrofa,* Cat. No. 071.96) and contain enough volume for 96 reactions. These Globin Block Modules can only be used with the QuantSeq 3′ mRNA-Seq Library Prep Kits from Lexogen.

Figure 1. Schematic overview of the QuantSeq workflow using Globin Block Modules for globin depletion. Whole blood total RNA contains a mixture of both globin and non-globin mRNAs, which are both reverse transcribed by oligo(dT) priming during first strand synthesis. The RS-Globin Block (RS-GB ●) solution is then added instead of the standard RNA Removal Solution (RS ○). Following RNA removal, the Globin Blocker oligos bind specifically to globin first strand cDNA downstream of random primers and block globin second strand cDNA synthesis. After second strand synthesis, the sample will contain both non-amplifiable globin cDNA fragments and tagged double-stranded cDNA library fragments for non-globin mRNAs. During PCR, only the non-globin library fragments are amplified to add full-length adapters and indices for Illumina sequencing.

2. Kit Components and Storage Conditions

Globin Block Modules 070/071, 96 preps (-20 °C)

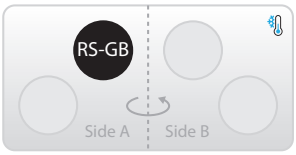

Figure 2. Location of kit components.Figure 2. Location of kit components. Each Globin Block Module contains one of the following Removal Solutions-Globin Block: *Homo sapiens* (Cat. No. 070) or *Sus scrofa* (Cat. No. 071).

Kit Component	Tube Label	Volume*	Storage
		96 rxn	
Removal Solution-Globin Block, *Homo sapiens*, 96 rxn (Cat. No. 070)	**RS-GBHs ●**	528 µl	-20 °C
Removal Solution-Globin Block, *Sus scrofa*, 96 rxn (Cat. No. 071)	**RS-GBSs ●**	528 µl	-20 °C

*including ≥10 % surplus

ATTENTION:

- The Globin Block Modules for QuantSeq **are not stand-alone kits**. They are Add-on Modules for the QuantSeq FWD (Cat. No. 015, 113 - 115, 129 - 131) and REV (Cat. No. 016) kits and require the components therein for functionality.
- The Removal Solution-Globin Block (**RS-GB ●**) replaces the standard RNA Removal Solution (**RS ○**) at the RNA removal step of the standard QuantSeq 3′ mRNA-Seq Library Prep protocol.

NOTE: For additional user-supplied consumables and equipment needs, please refer to the QuantSeq 3′ mRNA-Seq Library Prep Kit for Illumina User Guides.

3. Protocol

ATTENTION: QuantSeq generated first strand cDNA (FWD, Cat. No. 015, 113 - 115, 129 - 131 and REV, Cat. No. 016) is required as input for RNA removal using the Removal Solution-Globin Block (**RS-GB** ●, Cat. No. 070, 071). Globin blocker oligos hybridize specifically to Globin first strand cDNA and block the extension of second strand cDNA in subsequent library preparation steps. This results in non-amplifiable, single-stranded Globin cDNA fragments.

RNA Removal - Globin Block

During this step the RNA template is degraded, which is essential for efficient second strand synthesis, and globin blockers are added. Before removing the sealing foil after the first strand synthesis reaction, quickly spin down the plate to make sure all liquid is collected at the bottom of the wells.

ATTENTION: The Removal Solution-Globin Block (**RS-GB** ●) replaces the RNA Removal Solution (**RS** ○) from the standard QuantSeq 3' mRNA-Seq Library Prep Kits for Illumina.

NOTE: RS-Globin Block, *Homo sapiens* (**RS-GBHs** ●) should be used for human blood RNA libraries. RS-Globin Block, *Sus scrofa* (**RS-GBSs** ●) should be used for pig blood RNA libraries.

➡	First Strand cDNA Synthesis is carried out according to the standard QuantSeq User Guide (015UG009 or 113UG227) steps 1 to 4.
5	Add 5 µl of Removal Solution-Globin Block (**RS-GB** ●) directly to the first strand cDNA synthesis reaction. Mix well and reseal the plate using a fresh foil. **REMARK:** Use a pipette set to 15 µl for efficient mixing.
6	Incubate for 10 minutes at 95 °C, then cool down to 25 °C. Spin down the plate at room temperature and carefully remove the sealing foil.
➡	Proceed to Second Strand Synthesis (step 7) as described in QuantSeq User Guide (015UG009 or 113UG227).

4. Appendix A: Input RNA and PCR Cycles

Total RNA from blood is the intended input for QuantSeq library prep using RS-Globin Block solutions for globin depletion. No prior depletion of globin mRNA, poly(A) enrichment, or ribosomal RNA is required. The minimum recommended input for this protocol is 50 ng of total RNA from whole blood, or leukocyte-enriched blood samples (RIN ≥6). It is important to ensure that total RNA is of high purity: Ideal values for A260/280 and A260/230 absorbance ratios should be 1.8 - 2.1, and approximately 2, respectively. For low quality RNA samples, please follow the protocol modification listed at the bottom of the page.

We recommend the use of Lexogen's SPLIT RNA Extraction Kit (Cat. No. 008) for isolation of total RNA from fresh or frozen blood. Additional red blood cell lysis may be performed prior to RNA extraction to obtain leukocyte-enriched blood RNA. For protocol details please contact support@lexogen.com. Total RNA isolated from blood using the PAXgene® Blood RNA System (Tubes and Kit, Qiagen) is also compatible with this protocol. The table below shows an example of PCR cyle numbers used for input amounts of human and pig total RNA from blood.

Species	Whole Blood or Leukocyte Blood	Input Amount	PCR Cycles	
			Undepleted	+ RS-Globin Block
Human	Whole Blood	250 ng	13	14
		50 ng	15	16
	Leukocyte-enriched Blood	50 ng	16	17
		50 ng*	15	16
Pig	Whole Blood	100 ng	16	17

* Indicates RNA isolated using PAXgene® Blood System (Tubes and Kit, Qiagen), which includes 24-hour red blood cell lysis. Pig blood was collected in Tempus blood RNA tubes and extracted using the Preserved Blood RNA Purification Kit I (Norgen Biotek). All other RNA was extracted using the SPLIT RNA Extraction Kit (Cat. No. 008.48). All libraries were prepared with single indexing. Linker sequences are 122 bp including 6 nt long i7 indices.

Globin mRNAs constitute 50 - 80 % of the total mRNA pool. Therefore, when blocking these mRNAs from the final step of library generation, the cycle numbers need to be increased to achieve the same library yield as non-depleted samples. Adding one additional PCR cycle compared to non-depleted blood samples is typically sufficient. However, as the mRNA content, purity, and quality of total RNA from blood samples may vary depending on the origin of the sample. Therefore, **we strongly recommend performing the qPCR assay to determine the optimal number of cycles for the library amplification** (see the respective Appendix in the QuantSeq User Guides (015UG009 or 113UG227)).

ATTENTION: For low quality RNA we recommend: skipping step 2, reducing the amount of **PS** added in step 16 (48 µl instead of 56 µl), and reducing the amount of **PB** in step 29 (27 µl instead of 30 µl). For information regarding the use of input amounts <50 ng please contact support@lexogen.com, or see the QuantSeq FWD online FAQs at www.lexogen.com.

Typical Results

Libraries prepared from human blood RNA with standard QuantSeq Library Prep Kit and protocol display distinct peaks in bioanalyzer traces at 197 bp, 212 bp, 222 bp, 235 bp, and 312 bp, which correspond to abundant globin mRNA library fragments (HBA1, HBA2, and HBB). These peaks are reduced in libraries prepared with RS-Globin Block, *Homo sapiens* (**RS-GBHs ●**), as shown below in Figure 3 (50 ng whole blood total RNA).

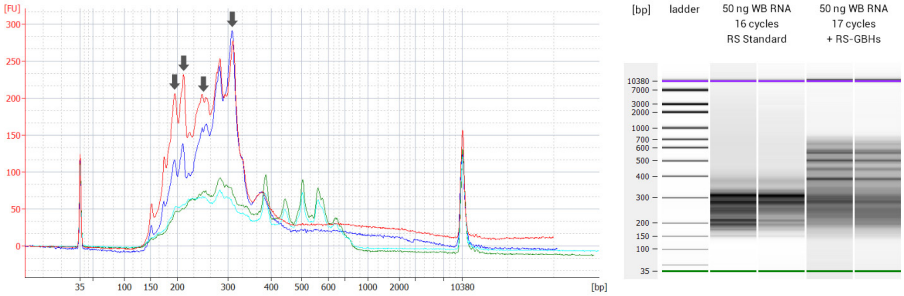

Figure 3. Bioanalyzer traces for human QuantSeq FWD libraries prepared with (+Globin Block) and without (Standard) the RS-Globin Block solution (RS-GBHs, Cat. No. 070.96). Replicate libraries were prepared from 50 ng of whole blood (WB) RNA with the Standard QuantSeq FWD protocol (blue and red traces), versus QuantSeq +Globin Block (green and turquoise traces). RNA was isolated using the SPLIT RNA Extraction Kit without red blood cell lysis (Lexogen). Grey arrows indicate major globin peaks reduced in +Globin Block Libraries.

Pig blood libraries prepared with RS-Globin Block, *Sus scrofa* (**RS-GBSs ●**) also show an altered peak profile compared to libraries prepared with standard QuantSeq RS (see Figure 4 below).

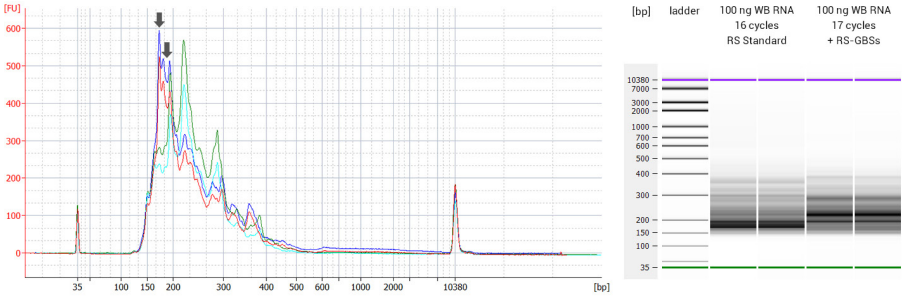

Figure 4. Bioanalyzer traces for pig QuantSeq FWD libraries prepared with (+Globin Block) and without (Standard) the RS-Globin Block solution (RS-GBSs, Cat. No. 071.96). Replicate libraries were prepared from 100 ng of whole blood (WB) RNA with the Standard QuantSeq FWD protocol (blue and red traces), versus QuantSeq +Globin Block (green and turquoise traces). RNA was isolated using the Preserved Blood RNA Purification Kit + DNase I Kit (Norgen Biotek). Grey arrows indicate major globin peaks reduced in +Globin Block Libraries.

Overcycling

A peak in high molecular weight regions (between 1,000 - 9,000 bp) is an indication of overcycling. This could occur if cycle numbers are increased too much to compensate for lower input material. Prevent overcycling by using the qPCR assay as described in the respective Appendix of the QuantSeq User Guides (015UG009 or 113UG227).

5. Appendix B: Revision History

Publication No. / Revision Date	Change	Page
070UG365V0101 Jan. 25, 2023	Updated Kit Components Figure 2 and Table to reflect current packaging and storage requirements.	6
070UG365V0100 Aug. 16, 2021	Initial Release.	

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Associated Products:

008 (SPLIT RNA Extraction Kit)
015 (QuantSeq 3′ mRNA-Seq Library Prep Kit for Illumina (FWD))
016 (QuantSeq 3′ mRNA-Seq Library Prep Kit for Illumina (REV) with Custom Sequencing Primer)
020 (PCR Add-on Kit for Illumina)
022 (Purification Module with Magnetic Beads)
025, 050, 051, 141 (SIRVs Spike-in RNA Variant Control Mixes)
080 (Reamplification Add-on Kit for Illumina)
081 (UMI Second Strand Synthesis Module for QuantSeq FWD (Illumina, Read 1))
113 - 115, 129 - 131 (QuantSeq 3′ mRNA-Seq Library Prep Kit FWD with UDI 12 nt Set A1, A2, A3, A4, A1-A4, or B1)

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