

NEXTFLEX® Universal Blockers

(Compatible with Illumina[®] and Element[®] Platforms)

KIT CONTAINS : 16, 48, or 96 RXNS

USER MANUAL FOR : #NOVA-5143231 #NOVA-5143232 #NOVA-5143233

NEXTFLEX® Universal Blockers

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This product is for research use only.

Not for use in diagnostic procedures.

This manual is proprietary to Revvity, Inc., and intended only for customer use in connection with the product(s) described herein and for no other purpose. This document and its contents shall not be used or distributed for any other purpose without the prior written consent of Revvity. Follow the protocol included with the kit.

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GENERAL INFORMATION

Product Overview

The NEXTFLEX[®] Universal Blockers are designed to prevent concatermerization of library molecules during target capture hybridizations via the adapter sequences that are added to all library molecules before enrichment. Each strand of these adapter sequences can hybridize with the complementary adapter sequence strand of another library molecule during this enrichment hybridization, creating a "daisy-chain" effect between off-target library molecules and the on-target library molecules that are intended targets of the capture probe set used in the enrichment hybridization. By blocking this complementary adapter sequence interaction, the number of on-target reads improves dramatically and the depth of enrichment increases.

The NEXTFLEX[®] Universal Blockers are designed for use with Illumina[®] indexed adapters. They are effective in blocking single-indexed or dual-indexed libraries, without being affected by index length or the presence of a unique molecular index (UMI). The NEXTFLEX[®] Universal Blockers are designed to target both strands of the adapter sequence regions.

The NEXTFLEX[®] Universal Blockers are compatible with ligation-based workflows, such as the libraries prepared with NEXTFLEX[®] Unique Dual-Indexed Barcodes. They are also compatible with tagmentation-based workflows, such as Illumina[®] Nextera[™] library prep kits.

Kit Contents, Storage and Shelf Life

The kits contain enough materials to prepare 8, 48, or 96 hybridization reactions. The shelf life is at least 12 months when stored properly. Store at -20° C.

| Kit Contents | Cap Color | Amount (8 rxn / 48 rxn / 96 rxn) | Storage Temp. |
|--|-----------|-------------------------------------|------------------|
| NEXTFLEX [®] Universal Blockers | GREY CAP | 4.8 / 29 / 58 μL | -20°C |

Warnings and Precautions

- We strongly recommend that you read the following warnings and precautions. Periodically, optimizations and revisions are made to the components and manual. Therefore, it is important to follow the protocol included with the kit. If you need further assistance, you may contact your local distributor, or contact us at <u>https://www.revvity.com/contact-us/technical-support</u> and choose the "Next Gen Sequencing" category.
- Do not use the kit past the expiration date.
- Ensure pipettes are properly calibrated as library preparations are highly sensitive to pipetting error.
- Try to maintain a laboratory temperature of 20°-25°C (68°-77°F).

| Version | Date | Description |
|---------|---------------|--------------------|
| V21.05 | May 2021 | Product Launch |
| V23.11 | November 2023 | Rebrand to Revvity |

Usage

The NEXTFLEX[®] Universal Blockers can be used with IDT[™] xGEN[™], Twist Bioscience, and Agilent[®] SureSelect hybridization capture libraries and workflows.

Recommendations:

IDT[™] xGEN[™] Hybridization Capture Libraries

Follow Standard protocol of xGEN[™] hybridization capture libraries. During hybridization step use 0.6 µL of NEXTFLEX[®] Universal Blocker per reaction, instead of 2 uL of xGEN[™] blocker, independent of library type (Illumina[®] Truseq[™] or Nextera[™] type) and index length and/or UMI.

| Blocker Master Mix Component | Volume per reaction |
|------------------------------|---------------------|
| Human Cot DNA | 5 µL |
| NEXTFLEX® Universal Blockers | 0.6 µL |
| TOTAL | 5.6 µL |

Twist Bioscience Hybridization Capture Libraries

Follow Standard protocol of Twist Bioscience hybridization capture libraries. During hybridization step use 0.6 µL of NEXTFLEX® Universal Blocker per reaction, instead of 8 µL of Twist Universal Blocker, independent of library type (Illumina® Truseq[™] or Nextera[™] type) and index length and/or UMI.

| Blocker Master Mix Component | Volume per reaction |
|--|---------------------|
| Dried Indexed Library Pool | - |
| Blocker Solution | 5 µL |
| NEXTFLEX [®] Universal Blockers | 0.6 µL |
| Nuclease-free Water | 7.4 µL |
| TOTAL | 13 µL |

Agilent® SureSelect Hybridization Capture Libraries

Follow Standard protocol of Agilent[®] SureSelect hybridization capture libraries. During hybridization step use 0.6 µL of NEXTFLEX[®] Universal Blocker per reaction, instead of 0.6 µL of Block 3, independent of library type (Illumina[®] Truseq[™] or Nextera[™] type) and index length and/or UMI.

| Blocker Master Mix Component | Volume per reaction |
|--|---------------------|
| SureSelect Indexing Block 1 | 2.5 µL |
| SureSelect Block 2 | 2.5 µL |
| NEXTFLEX [®] Universal Blockers | 0.6 µL |
| TOTAL | 5.6 µL |



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