Notices

Limitations of use

For research use only. Not for use in diagnostic procedures. Unless otherwise agreed to in writing, IDT does not intend these products to be used in clinical applications and does not warrant their fitness or suitability for any clinical diagnostic use. Purchaser is solely responsible for all decisions regarding the use of these products and any associated regulatory or legal obligations.

Safety data sheets pertaining to this product are available upon request.

Safety Notices

Reminder symbols call attention to minor details that may be easily overlooked and compromise the procedure resulting in decreased assay performance.

Caution symbols denote critical steps in the procedure where risk of protocol failure or damage to the product itself could occur if not carefully observed.

Stop symbols indicate where this procedure may be safely suspended and resumed at a later time without risk of compromised assay performance. Make note of these steps and plan your workflow accordingly.

Contents

Revision history

Document Number	Date	Description of change
RA-DOC-620/REV01	September 2025	• Initial release.
RA-DOC-620/REV02	October 2025	• Added Illumina MiSeqi100 sequencer to the intended use section. • Updated RA-DOC-054 reference with a specific protocol step • Added part numbers in kit table

Technical support

Contact us directly at archer-tech@idtdna.com.

Overview

Intended use

The Archer Sequencing Accessory Kit for Illumina® is a sequencing pretreatment product. It is intended for research applications in conjunction with Archer reagent kits and corresponding target-enrichment panels to produce high-complexity libraries for use with Illumina next-generation sequencing (NGS) platforms which utilize patterned flow-cells.

Sequencing pretreatment maximizes the sequencing output of Archer libraries on Illumina patterned flow-cell instruments, specifically the Nextseq2000, and MiSeq i100. Libraries should be prepared per the appropriate Archer library preparation protocol. Individual libraries should be quantified and pooled per Quantify, Normalize, and Sequence Protocol for Illumina (RA-DOC-054). The sequencing pool is then diluted and used as input for the sequencing pretreatment step.

Archer reagents include:

- Archer Sequencing Accessory Kit for Illumina reagents are in liquid format.

Archer Sequencing Accessory Kit for Illumina (10029962)

Store at -30°C to -10°C

Materials Supplied		
Description	**Part Number**	**Quantity**
ILMN Sequencing Accessory Buffer	*10029963*	*1 tube (8 reactions)*
ILMN Sequencing Accessory Primer	*10029964*	
ILMN Sequencing Accessory Enzyme	*10029965*	

Materials required, but not supplied have been optimized for proper application of the Archer Library preparation. Use of other materials have not been tested by IDT.

Materials Required, but not Supplied		
Description	**Supplier**	**Part Number**
AMPure® XP beads	*Beckman Coulter*	*A63880*
1 M Tris-HCl, pH 8.0 (molecular biology grade)	*Various*	-
Ultrapure water (molecular biology grade)	*Various*	-
100% ethanol (ACS grade)	*Various*	-
RNase AWAY™	*Thermo Fisher Scientific*	*7003*
KAPA Universal Library Quantification Kit	*KAPA Biosystems*	*KK4824*
Standard PCR thermal cycler	*Various*	-
Real-Time PCR thermal cycler	*Various*	-
qPCR tubes	*Various*	-
0.2 mL PCR tubes	*Various*	-
DynaMag™-96 Side Magnet	*Thermo Fisher Scientific*	*12331D*
Microcentrifuge	*Various*	-
Plate centrifuge	*Various*	-
Pipettes (P10, P20, P200 and P1000)	*Pipetman or equivalent*	-
Sterile, nuclease-free aerosol barrier pipette tips	*Various*	-
Vortex mixer	*Various*	-
PCR tube cooling block	*Various*	-
Gloves	*Various*	-

Before getting started

Important precautions

- **Read through the entire protocol before starting your library preparation.**
- Take note of safe stopping points throughout the protocol where samples can be safely frozen (-30° C to -10° C) to plan your workflow.
- Use good laboratory practices to prevent contamination of samples by PCR products.
- Use nuclease-free PCR tubes, microcentrifuge tubes, and aerosol-barrier pipette tips.
- Wipe down workstation and pipettes with nuclease and nucleic acid cleaning products (e.g., RNase AWAY, Thermo Fisher Scientific).
- Verify that the thermal cycler used for library preparation is in good working order and currently calibrated according to manufacturer specifications. When using a thermal cycler with a 100% ramp rate >6°C/sec, set the ramp rate to go no higher than 6°C/sec.
- Reaction cleanup with AMPure XP beads (Beckman Coulter) is performed at room temperature (20°C to 25°C) and is used repeatedly throughout the workflow. Ensure that AMPure XP beads are equilibrated to room temperature and fully resuspended by vortexing until homogenous in both color and appearance prior to drawing out material for **each** use.

Working with liquid reagents

- Archer Sequencing Accessory Kit enzymes are sensitive to frequent temperature changes and potential freeze-thaw events.
- Enzymes will not freeze when stored at recommended storage temperature range but may freeze during shipment.
- For best results, it is recommended that you store the enzymes provided in a -20°C benchtop cooler box and use this to transport enzymes between workspaces and freezers.
- Buffer mixes will freeze at recommended storage temperature and should be thawed on ice prior to use. For best results, buffer mixes should be aliquoted to appropriate batch sizes for single use with adequate overage, such that aliquots are not re-frozen after thawing.

Input nucleic acid

- Archer libraries pooled in EDTA-free buffer (pH 7-8) is the optimal starting template for the sequencing pretreatment. Do NOT use EDTA-containing buffers.

Reagents to prepare before starting

- Make at least **10 mL** fresh 10 mM Tris-HCl, pH 8.0 from 1M Tris-HCl, pH 8.0 and ultrapure water.
  - Mix **100 µL** 1M Tris-HCl, pH 8.0 with **9,900 µL** ultrapure water.
  - 10 mM Tris-HCl, pH 8.0 can be used for up to one week after mixing.

- Make at least **50 mL** fresh 70% ethanol from 100% ethanol and ultrapure water.
  - Add **35 mL** 100% ethanol to **15 mL** ultrapure water.
  - Tightly close the cap to minimize evaporation when not in use.
  - 70% ethanol is appropriate for use for up to one week after mixing.

Protocol

Sequencing Pretreatment

1. Dilute library pool to 3nM using 10 mM Tris-HCl, pH 8.0. Keep diluted pool on ice.
2. Thaw the **ILMN Sequencing Accessory Buffer** (10029963) and **ILMN Sequencing Accessory Primer** (10029964), on ice. Vortex the tubes, then spin down.
3. On ice, make a **Sequencing Pretreatment Master Mix** working solution based on the number of pools (including but not exceeding 20% overage) using the table below.

Sequencing Pretreatment Master Mix	1X (µL)	___X (µL)
ILMN Sequencing Accessory Buffer (10029963)	11.2	
ILMN Sequencing Accessory Primer (10029964)	4.0	
ILMN Sequencing Accessory Enzyme (10029965)	0.8	
Nuclease-free water	16.5	
Total volume	*32.5*	

   a. Mix by briefly vortexing and spin down.

   b. Keep master mix on ice.

4. To new 0.2mL 8-strip tubes or plate wells add:

   a. **32.5 µL** Sequencing Pretreatment Master Mix.

   b. **7.5 µL** of 3nM diluted library pool.

5. Mix by briefly vortexing and spin down.
6. Return tubes to ice.
    a. Transfer reactions to a thermal cycler and initiate an incubation using the following program and guidelines:
    b. Use a heated lid (≥100°C).

Sequencing Pretreatment Master Mix incubation conditions

Step	Temperature (°C)	Time	Cycles
1	95	3 min	1
2	95	15 sec	2
3	65	1 min	
4	72	1 min	
5	72	3 min	1
6	4	Hold	1

Reaction Cleanup after Sequencing Pretreatment

Refer to Important Precautions section for guidance on working with AMPure XP beads.

1. Completely resuspend AMPure XP beads by vortexing.
2. Add **1.2X** volume (**48 µL**) of AMPure XP beads to each sequencing pretreatment reaction.
3. Vortex well or pipette 10 times to mix and visually inspect the color of the sample to ensure a homogenous mixture.
4. Incubate for **5 minutes** at room temperature (20ºC to 25°C).
5. Briefly spin down tubes.
6. Place tubes on the magnet for **4 minutes or until beads are fully pelleted** against the tube wall.
7. Without disturbing the bead pellet, use a pipette to remove and discard the supernatant. If the pellet becomes dislodged from the magnet and a portion is drawn into the pipette tip, return contents to the tube and repeat magnet incubation step.
8. Wash beads **two times** with 70% ethanol while still on the magnet. For each wash:
    a. Add **200 µL** 70% ethanol.
    b. Incubate for **30 seconds** at room temperature (20°C to 25°C).

c. Carefully remove ethanol and discard.

9. After the final wash, use a pipette (≤20 µL capacity) to completely remove visible supernatant residue and allow tubes to dry for **3-5 minutes** at room temperature with open lids. **Take care not to over-dry beads** as this will significantly decrease overall recovery (yield) of nucleic acid.
10. Elute DNA by resuspending beads in **20 µL** 10 mM Tris-HCl, pH 8.0.
11. Place tubes back on the magnet for **2 minutes**.
12. Transfer **18 µL** of the purified solution to a new 0.2mL PCR tube. Be sure to avoid transferring beads to the fresh tube.
13. Stop or proceed directly to Sequencing.

Safe stopping point: It is okay to stop and store the reactions at -30°C to -10°C.

Quantify and Sequence

Proceed to quantification of the final pool and sequencing starting at step 6 of the Quantify, Normalize, and Sequence Protocol for Illumina (RA-DOC-054).