Ion AmpliSeq™ Direct FFPE DNA Kit

Catalog Numbers A31133, A31136

Pub. No. MAN0014881 **Rev.** C.0

Kit contents

The Ion AmpliSeq™ Direct FFPE DNA Kit (Cat. No. A31133 – 8 preparations, or Cat. No. A31136 – 96 preparations) provides sufficient reagents for sample preparation of DNA from unstained, slide-mounted, formalin-fixed, paraffin-embedded (FFPE) tissue samples for downstream library preparation without nucleic acid isolation or quantification.

Component	Cat. No. A31133 (8 preparations)	Cat. No. A31136 (96 preparations)	Storage
Transfer Solution (purple cap)	240 µL	3 × 960 µL	2°C to 8°C
Direct Reagent (orange cap)	170 µL	3 × 675 µL	

Required materials and equipment

Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

Description	Source
One of the following: • GeneAmp™ PCR System 9700 • Veriti™ 96-Well Thermal Cycler • ProFlex™ 96-well PCR System	See web product pages
MicroAmp™ Optical 96-Well Reaction Plate	N8010560 4306737 (with barcode)
MicroAmp™ Clear Adhesive Film	4306311
MicroAmp™ Optical Film Compression Pad	4312639
Eppendorf™ DNA LoBind™ Microcentrifuge Tubes (0.5-mL or 1.5-mL)	MLS
Pipettors, 2–200 µL, and low-retention filtered pipette tips	MLS

Prepare reagents

- Equilibrate Transfer Solution to room temperature (15–30°C) before use.
- Keep Direct Reagent on ice prior to use.

Prepare FFPE DNA

The recommended tissue area to be used for this protocol is 4–100 mm^2 from a 5–10 µm thick unstained section mounted on a slide. The shaded squares (Figure 1) represent 4 mm^2 area out of a total gridded area of 100 mm^2. Deparaffinization is not required. If desired, scrape unwanted tissue from the slide before transfer.

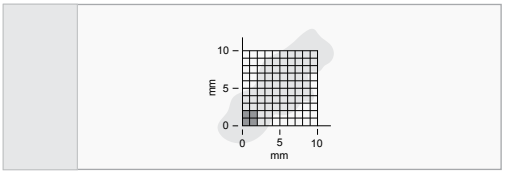

Figure 1 Standard microscope slide, 25 × 75 mm.

1. For each sample, pipet 30 µL of Transfer Solution into a single well of a 96-well PCR plate.

   Note: Transfer Solution is viscous, pipet slowly.

   Note: Alternatively, label one nuclease-free 1.5-mL Eppendorf LoBind™ tube for each FFPE tissue sample and perform incubations in a heated block.

2. Using a single 20-µL pipette tip for each sample:
   a. Pipet 2–10 µL of the Transfer Solution from the well onto the region of interest of the FFPE tissue section mounted on a slide.
   b. Using the same 20-µL pipette tip, spread the Transfer Solution to ensure complete coverage of the region of interest, then scrape and break up the tissue with the pipette tip. The tissue should be a slurry of fine particles in the Transfer Solution.
3. Pipet the slurry from the slide back into the same well of the 96-well plate containing Transfer Solution.
4. Pipette the slurry up and down at least five times, leaving as much tissue as possible in the 96-well plate.
5. If needed, use the same tip to repeat steps 2–4, transferring as much of the region of interest as possible into the 96-well plate.

   Note: The final volume of Transfer Solution remaining in the 96-well plate may vary, but no volumetric adjustment is required.
6. Add 21 µL of Direct Reagent to each well containing sample in the 96-well plate.
7. Set a pipette to 30 µL, then mix the Direct Reagent and slurry by pipetting up and down ten times.
8. Seal the plate with a MicroAmp™ Adhesive Film, then verify that the contents are at the bottom of each well of the 96-well plate.

   Note: If necessary, gently tap the plate on a hard flat surface to collect the contents at the bottom of the wells.
9. Place a compression pad on the plate, load the plate into the thermal cycler, then run the following program:

Temperature	Time
65°C	15 min
20°C	Hold (for up to 30 minutes)

10. Proceed directly to the following section.

Remove aliquot for library preparation

Libraries may be prepared using either automated or manual library preparation procedures.

Note: The FFPE DNA preparation can be stored for up to 6 months at –20°C before library preparation.

- Manual protocol

  See the *Ion AmpliSeq™ Library Kit 2.0 User Guide* (Pub. No. MAN0006735) for more information.

  a. Set a 20-µL pipette to 15 µL, depress the plunger to the first stop, and insert the pipet tip below the interface between phases (Figure 2, left), then pipet the lower (aqueous) phase up and down to mix the sample.

     Note: Mixing the sample prior to removal ensures a homogenous sample before removing aliquots. Avoid pipetting the upper phase and interface while mixing.

  b. Aspirate 6–12 µL —depending on the number of primer pools and reaction size— of the lower phase (Figure 2, right), then transfer the sample to the appropriate well of a 96-well PCR plate.

     Note: If you are not quantifying sample DNA concentration, use the maximum volume of DNA in the target amplification reaction. See "**(Optional) Qubit™ Fluorometer: Quantify the FFPE genomic DNA**" in the *Ion AmpliSeq™ Library Kit 2.0 User Guide* (Pub. No. MAN0006735) for more information.

  c. Proceed to "**Prepare DNA target amplification reactions**" in the *Ion AmpliSeq™ Library Kit 2.0 User Guide* (Pub. No. MAN0006735).

- Automated protocol

  See "**Remove aliquot for library preparation**" in the *Ion AmpliSeq™ Library Preparation on the Ion Chef™ System User Guide* (Pub. No. MAN0013432) for more information.

  a. Set a 20-µL pipette to 15 µL, depress the plunger to the first stop, and insert the pipet tip below the interface between phases (Figure 2, left), then pipet the lower (aqueous) phase up and down to mix the sample.

     Note: Mixing the sample prior to removal ensures a homogenous sample before removing aliquots. Avoid pipetting the upper phase and interface while mixing.

  b. Aspirate 15 µL of the lower phase (Figure 2, right), then transfer the sample to the appropriate well of the IonCode™ 96-well PCR plate.

  c. Proceed immediately to "**Add DNA to the IonCode™ Barcode plate**" in the *Ion AmpliSeq™ Library Preparation on the Ion Chef™ System User Guide* (Pub. No. MAN0013432).

IMPORTANT! Start the Ion Chef™ library preparation run within 10 minutes of transferring the last sample to the IonCode™ 96-well PCR plate. If ≥10 minutes has elapsed, pipet each sample up and down at least 5 times to mix, then load the IonCode™ 96-well PCR plate onto the Ion Chef™ and start the run.

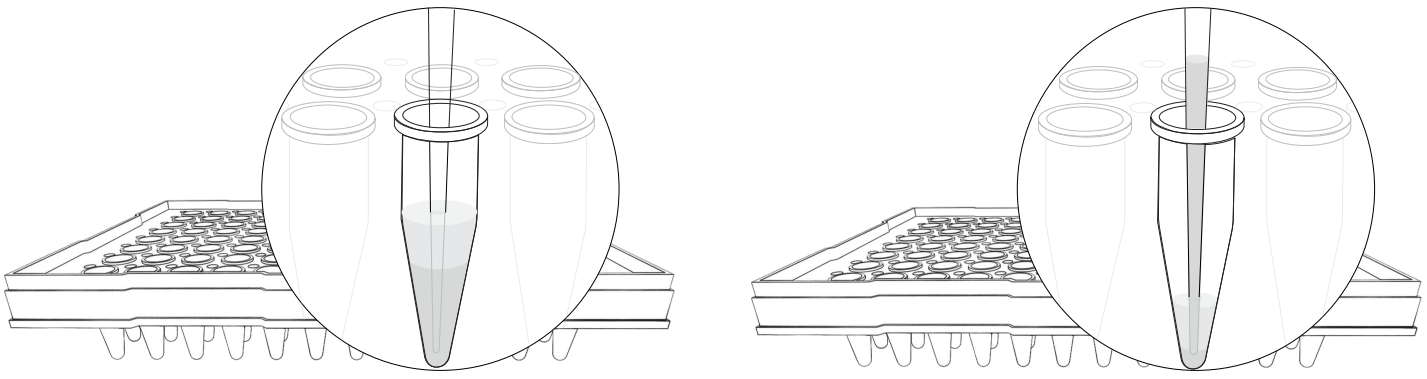

Figure 2 Remove aliquot.

IMPORTANT! Avoid pipetting the upper layer that contains the Transfer Solution. Carefully inspect each transferred sample aliquot for air bubbles. Remove any air bubbles by gently pipetting up and down.

Appendix A Troubleshooting

Observation	Possible cause	Recommended action
Tissue does not transfer from slide	Not enough Transfer Solution.	Hold the slide at a 45° angle and pipet extra Transfer Solution to the top of the slide allowing the tissue to flow towards the bottom. Remove collected tissue from the bottom, repeat as needed.
	Tissue is clumpy.	Transfer the mass of tissue to a collection tube, then continue breaking it up with a pipet tip.
		Pre-incubate with Transfer Solution on slide for 5 minutes, then proceed to scraping.
Difficulty scraping tissue off the slide	Tissue is fibrous.	Scrape with 200-µL tip prior to transfer, using a circular motion, then continue with a 20-µL tip.
		Scrape and homogenize tissue with a scalpel blade, then continue breaking up tissue with a 20-µL tip.
		Repeat transfer process with a larger volume of Transfer Solution.
	Target tissue area is surrounded by undesired tissue.	Use a scalpel blade to scrape away undesired tissue or paraffin, then use Transfer Solution to collect the desired tissue.
Excess undissolved tissue in Direct Reagent	Too much tissue in reaction.	Use 4–100 mm^2 tissue section. Tissue sections should be 5–10 µm thick.
	Digest may be incomplete.	Incubate for an additional 5–15 minutes at 65°C. After digestion sample may still be cloudy, this will not affect performance. Ensure homogenous mixing of the sample prior to removing an aliquot for target amplification. Undissolved tissue that can be aspirated with a pipet tip may still be added to the Target Amplification reaction.
		Centrifuge at ≥1,000 × *g* for 1 minute, then transfer 15 µL to a fresh tube, avoiding the fibrous pellet.
Transfer Solution and Direct Reagent do not separate into two phases	Too much paraffin in sample.	Use a scalpel blade to scrape away undesired paraffin prior to adding Transfer Solution to the desired tissue area.
		Centrifuge at ≥1,000 × *g* for 1 minute, then transfer 15 µL to a fresh tube, avoiding the fibrous pellet and tube walls.
		Perform partial deparaffinization before adding Transfer Solution to tissue on the slide: 1. Submerge slide in 100% xylene for 30 seconds. 2. Remove the slide, then drain any excess xylene. 3. Submerge slide in 100% ethanol for 30 seconds. 4. Remove the slide, then allow to air dry.
Difficulty transferring lower (aqueous) phase to target amplification reaction	Transfer Solution is in pipet tip.	Return tip contents to reaction tube, then centrifuge at ≥1,000 × *g* for 1 minute to separate phases. Move pipet quickly through the upper phase when transferring. **Note:** Transfer Solution will not interfere with target amplification.
Low AmpliSeq™ library concentration	Insufficient tissue was used.	Use 25–100 mm^2 tissue section of 5–10 µm thickness. If needed, use multiple slides to obtain 25–100 mm^2 tissue.
	Insufficient amplifiable DNA was used.	FFPE DNA quality may vary due to tissue fixation methods, length of storage time, and storage conditions. Although the Qubit™ assay may detect the presence of DNA, the DNA may not be of sufficient quality to generate an AmpliSeq™ library. Re-prepare FFPE DNA from 100 mm^2 tissue section of 5–10 µm thickness. If needed, use multiple slides to obtain 100 mm^2 tissue.
	Inhibitors are present in the tissue.	Inhibitors such as high melanin content can affect PCR, reduce the volume of input sample going into the target amplification reaction.
Qubit™ result indicates high concentration (>10 ng/µL)	FFPE tissue has high DNA content.	Reduce the volume of input sample going into the target amplification reaction by one half to one quarter.
Qubit™ result indicates low concentration (<0.5 ng/µL) Samples with low DNA yield can still be sufficient to generate an AmpliSeq™ library.	FFPE tissue has low DNA content.	Increase the number of target amplification cycles by 2 or 3.

Appendix B Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the "Documentation and Support" section in this document.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

Documentation and support

Related documentation

Document	Publication number	Description
Ion AmpliSeq™ Library Kit 2.0 User Guide	MAN0006735	Describes the preparation of Ion AmpliSeq™ libraries for sequencing on the Ion PGM™, Ion Proton™, and Ion GeneStudio S5 Series[1] Sequencers.
Ion AmpliSeq™ Library Preparation on the Ion Chef™ System User Guide	MAN0013432	Describes the automated preparation of Ion AmpliSeq™ libraries using the Ion Chef™ System for sequencing on the Ion PGM™, Ion Proton™, and Ion GeneStudio S5 Series[1] Sequencers.

[1] Ion GeneStudio S5 Series Sequencer refers generically to the three Ion GeneStudio S5 Sequencers or Systems (Ion GeneStudio S5 (Cat. No. A38194), Ion GeneStudio S5 Plus (Cat. No. A38195), and Ion GeneStudio S5 Prime (Cat. No. A38196)). The Ion S5™ Sequencer and the Ion S5™ XL Sequencer are no longer available for purchase and are replaced by the Ion GeneStudio S5 Sequencer and Ion GeneStudio S5 Prime Sequencer, respectively. Both of the Ion S5™ and Ion S5™ XL Sequencers continue to be supported by Thermo Fisher Scientific.

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- Product documentation, including:
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

  Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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Revision history: Pub. No. MAN0014881

Revision	Date	Description
C.0	12 March 2018	• Removed the reference for the discontinued AB™ Applied Biosystems™ 2720 Thermal Cycler. • Updated for the Ion GeneStudio S5 Series Sequencer.
B.0	18 April 2017	Update Direct FFPE DNA preparation storage to 6 months at –20°C.
A.0	18 May 2016	Instructions for sample preparation of DNA from FFPE tissue samples for subsequent Ion AmpliSeq™ library preparation.