thermo scientific | USER GUIDE

KingFisher™ Ready DNA Ultra 2.0 Prefilled Plates

Isolation of DNA from buccal swab, buffy coat, saliva, or whole blood using prefilled single use kits for automation

Catalog Number A36586

Pub. No. MAN0018361 **Rev.** B.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

The Thermo Scientific™ KingFisher™ Ready DNA Ultra 2.0 Prefilled Plates are developed for scalable, rapid purification of high-quality DNA from a variety of sample matrices. DNA purified with this kit can be used in a broad range of molecular biology downstream applications, such as sequencing, genotyping, and qPCR. This protocol guides users through automated isolation of DNA from buccal swab, buffy coat, saliva, or whole blood using the KingFisher™ Flex magnetic particle processor.

Contents and storage

Reagents that are provided in each kit are sufficient for 96 reactions.

IMPORTANT! On receipt, store all plates and reagents in an upright position at room temperature.

Table 1 KingFisher™ Ready DNA Ultra 2.0 Prefilled Plates

Component	Quantity	Storage
Empty sealed 96 Deep well plate for sample input and digestion	1	Store upright at 15°C to 25°C
96 deep well plate filled with Binding Solution	1	
96 deep well plate filled with Magnetic Beads	1	
96 deep well plate filled with Wash I Solution	1	
96 deep well plate filled with Wash II Solution_1	1	
96 deep well plate filled with Wash II Solution_2	1	
96 deep well plate filled with Elution Solution	1	
Enhancer Solution	1 bottle	
Proteinase K Solution	1 bottle	
96 Deep well Tip comb Nested in a 96 Deep Well plate	1	

Required materials not supplied

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

Table 2 For all protocols

Item	Source
Instrument	
KingFisher™ Flex Magnetic Particle Processor 96DW with 96 deep-well head and a 96 deep well heating block (not a standard heating block)	5400630
Equipment	
P1000 12 channel multichannel pipette[1]	MLS
P10 pipette[1]	MLS
P100/P200 pipette[1]	MLS

[1] Electronic multichannel / multidispense pipettes are not recommended for handling viscous solutions or sample types.

Table 3 For buccal swab protocol

Item	Source
Incubator with metal racks	MLS
Plate shaker[1], capable of shaking plates at a minimum of 900 rpm	88880023
Materials	
96 DW plate for digestion	95040450
MicroAmp™ Clear Adhesive Film	4306311
4N6FLOQSwabs™, regular tip	4473979
Reagents	
Nuclease-Free Water	AM9932
Phosphate Buffered Saline (PBS (1X), pH 7.4)	10010023

[1] For digestion in a plate, not required for digestion in tubes

General guidelines

- The plates provided in this kit are single use plates only. Do not reuse the plates.
- Perform all steps at room temperature (15–25°C) unless otherwise noted.

For Research Use Only. Not for use in diagnostic procedures.

ThermoFisher SCIENTIFIC

- Precipitates and high viscosity can occur if plates or solutions are stored in a refrigerator or when the room temperature is too cold. If there are precipitates in these solutions, warm them at 37°C and gently mix to dissolve precipitates. Avoid creating bubbles.
- Yellowing of the Lysis/Binding and Wash I Solution is normal and will not impact buffer performance.

Guidelines for buccal swab protocol

- Equilibrate buccal swabs to room temperature to maximize DNA recovery.
- Cover the plate during the incubation and shaking steps to prevent spill-over and cross-contamination. The same MicroAmp™ Clear Adhesive Film can be used throughout the procedure, unless it becomes contaminated.
- If using a plate shaker other than the recommended shaker, verify that:
  a. The plate fits securely on the plate shaker.
  b. The recommended speeds are compatible with the plate shaker (Ideal shaker speeds allow for thorough mixing without splashing).
- *(Optional)* To prevent evaporation and contamination, cover the digestion plate with paraffin film or MicroAmp™ Clear Adhesive Film until they are loaded into the instrument.

Buccal swab sample collection and storage

IMPORTANT! Use the recommended swab with a foam tip. Use of cotton or generic polyester swabs may result in lower DNA yields or DNA that contains PCR inhibitors.

1. Have test subjects thoroughly rinse their mouths with water and swallow prior to swabbing.
2. Remove swab from packaging and thoroughly swab both cheeks of the test subject for 30 seconds each to maximize collection of buccal cells.
3. If necessary, store buccal swabs in the original pouch.

   IMPORTANT! Do not store buccal swabs in plastic tubes. Bacterial growth in sealed plastic tubes can cause DNA degradation.

Buccal swabs can be stored for up to 3 weeks at –20°C to 20°C before isolation.

Before each use of the kit

- Ensure plates are stored upright for 24 hours before opening.
- Gently mix reagents in bottles before use. Avoid creating bubbles.
- Flick downward or gently tap each plate before removing the seal to ensure reagents are in the bottom of the wells and not clinging to the underside of the seal. A brief centrifugation may be performed if desired but is not required.
- To remove seals from prefilled plates, place the prefilled plate squarely onto the benchtop, secure the plate with one hand and grasp the seal at the lower left corner with the other hand. Using a gentle but steady motion, peel the seal off of the plate diagonally toward the upper right corner without jostling the contents.

  IMPORTANT! If the seal begins to delaminate during peeling, stop, then rotate the plate 180 degrees and start peeling again from the edge that is now on the bottom left.

Perform DNA purification from buffy coat, saliva, or whole blood using KingFisher™ Flex

IMPORTANT! Do not attempt to process more than the maximum volume allowed for each sample type. Yields and quality will be reduced.

Sample type	Minimum sample input	Maximum sample input
Buffy coat	50 µL	200 µL
Saliva	150 µL	400 µL
Whole blood	50 µL	400 µL

1 Set up the instrument

a. Ensure that the instrument is set up with the proper magnetic head and the proper heat block, as indicated in the following table.

Component	Type
Magnetic head	96 Deep well magnetic head
Heat block	96 deep well heat block

IMPORTANT! Failure to use the proper magnetic head and heat block results in lower yields.

b. Ensure that the proper program (**FlexReadyDNA_Ultra2_96**) has been downloaded from the product page and loaded onto the instrument.

2 Prepare Sample Plate and digest with Proteinase K

a. Remove the Sample Input Plate from the box.

b. Remove the seal from the empty Sample Input Plate (for instructions on removing plate seals, see “Before each use of the kit“ on page 2).

This is the Sample Input Plate which will be slotted into deck position 1 on the Flex instrument after Enhancer, sample and Proteinase K is added.

c. Transfer appropriate amount (according to the following table) of Enhancer Solution first, then sample, and lastly Proteinase K, to the appropriate wells of Sample Input Plate.

Enhancer Solution (µL)	Sample Volume (µL)	Proteinase K (µL)
5	50	5
10	100	10
15	150	15
20	200	20
30	300	30
40	400	40

Note:
- Do not pre-mix the Enhancer Solution and Proteinase K due to potential inactivation of the Proteinase K.
- Do not change the order of pipetting.
- We recommend adding Enhancer and Proteinase K solutions using a 12-channel pipette for faster and easier dispensing.
- Once all components are added, proceed immediately to the instrument processing. There is no need for manual mixing beforehand if reagents and sample are added in the order as instructed.

d. Remove the seals from plates 2 to 6, then immediately load the remaining plates onto the instrument as prompted.

Note: Do not remove seal from lysis/binding solution plate 8, as this will not be loaded onto the instrument. We recommend removing the seals on a benchtop within close proximity to the instrument to reduce risk of losing reagents during transport from working bench to instrument.

2 Prepare Sample Plate and digest with Proteinase K *(continued)*

e. Confirm deck positions with the following table, then start the run.

Note: KingFisher™ Flex deck positions are also denoted on labels on the short and long ends of the plate.

Plate	Deck position	Prefilled plate	Contents	Prefilled volume
1	1	Sample input	Sample	Empty
2	2	Magnetic beads	Magnetic beads + Water	40 µL beads + 160 µL nuclease free H_2O
3	3	Wash I	Wash I	1,000 µL
4	4	Wash II_1	Wash II	1,000 µL
5	5	Wash II_2	Wash II	500 µL
6	6	Elution Plate	Elution Solution	100 µL
7	7	Tip comb	Tip comb	96 deep well tip comp nested in a 96 deep well plate
8	Don't put on deck	Lysis/Binding Buffer	Lysis/Binding Buffer	500 µL Lysis/Binding Solution

3 Purify the gDNA

a. After the 20 minute on-board digest, the instrument will pause and present the sample plate in the loading position. Remove the seal from Plate 8, then use a multichannel pipette to transfer 400 µL of the Lysis/Binding Solution from each well in Plate 8 to the corresponding well in Plate 1 (Sample Input Plate).

Note: There is no need to mix.

b. Immediately place the plate back onto deck position 1 on the instrument, then press **Start**.

The remainder of the run takes approximately 32 minutes.

c. At the end of the run, immediately remove the Elution Plate (Plate 6) from the instrument, then transfer the eluate to the final tube/plate of choice for final storage.

The purified DNA is ready for immediate use. Store eluted DNA at –20°C for long-term storage.

Perform DNA purification from buccal swab using KingFisher™ Flex

1 Prepare samples and digest with Proteinase K

Equilibrate buccal swabs to room temperature, before performing isolation, to maximize DNA recovery.

a. Place one swab, swab-head down, into a clean, empty 96 deep well plate or microfuge tube for digestion (not provided).

   Note: When a higher concentration of DNA is required, process two swabs in one well and proceed with the isolation as indicated.

b. Break enough of the stick off the swab so that the swab sits in the well without protruding.

   The recommended swabs have an easy break point, below the swab, that appears as a slight indentation in the stick portion of the swab.

c. Prepare sufficient Proteinase K Mix according to the following table, then gently invert or pipet up and down several times to thoroughly mix components.

Component [1]	Volume per well	Volume per plate (96 samples)
Enhancer Solution	40 µL	4.4 mL
PBS[2]	400 µL	44 mL
Proteinase K	40 µL	4.4 mL
Total volume	**480 µL**	**52.8 mL**

[1] Pipet the components in the order they are listed in the table.
[2] Nuclease free water can be used instead of PBS.

d. Add 480 µL of the Proteinase K Mix to each well containing a swab.

   Be careful to avoid touching the pipette tip to the swab when pipetting the Proteinase K Mix into the sample wells.

e. Seal the plate, then shake the sealed plate at 900 rpm for 5 minutes.

f. Take the plate off the plate shaker, then immediately incubate at 65°C for ≥ 20 minutes.

   IMPORTANT! Arrange plate in the incubator to allow adequate flow around the plate wells to ensure that samples quickly reach and maintain the incubation temperature.

g. Remove the sealed Sample Input Plate from the box, then unseal.

h. Transfer 480 µL of the lysate, one row at a time, directly to the Sample Input Plate provided in the kit.

   Note: Ensure each well contains 420–480 µL after transfer.

2 Set up the instrument

a. Ensure that the instrument is set up with the proper magnetic head and the proper heat block, as indicated in the following table.

Component	Type
Magnetic head	96 Deep well magnetic head
Heat block	96 deep well heat block

   IMPORTANT! Failure to use the proper magnetic head and heat block results in lower yields.

b. Ensure that the proper program (**FlexReadyDNA_Ultra2_Buccal96**) has been downloaded from the product page and loaded onto the instrument.

3 Prepare Sample Plate and purify the DNA

a. Remove the seal from Plate 8, then use a multichannel pipette to transfer 400 µL of the Lysis/Binding Solution from Plate 8 to Sample Input Plate.

Note: There is no need to mix.

b. Remove the seals from plates 2 to 6, then immediately load the remaining plates onto the instrument as prompted.

c. Confirm deck positions with the following table, then start the run.

Note: KingFisher™ Flex deck positions are also denoted on the plate labels located on short and long ends of the plate.

Plate	Deck position	Prefilled plate	Contents	Prefilled volume
1	1	Sample input	Sample	Empty
2	2	Magnetic beads	Magnetic beads + Water	40 µL beads + 160 µL nuclease free H_2O
3	3	Wash I	Wash I	1,000 µL
4	4	Wash II_1	Wash II	1,000 µL
5	5	Wash II_2	Wash II	500 µL
6	6	Elution Plate	Elution Solution	100 µL
7	7	Tip comb	Tip comb	96 deep well tip comp nested in a 96 deep well plate
8	Don't put on deck	Lysis/Binding Buffer	Lysis/Binding Buffer	500 µL Lysis/Binding Solution

Note: There is no need to mix.

The remainder of the run takes approximately 32 minutes.

d. At the end of the run, immediately remove the Elution Plate (Plate 6) from the instrument, then transfer the eluate to the final tube or plate of choice for final storage.

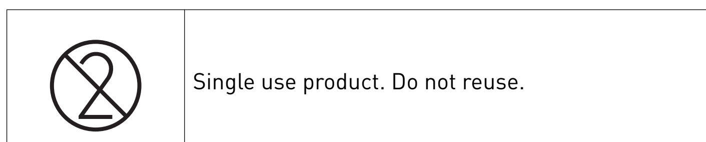
The purified DNA is ready for immediate use. Store eluted DNA at –20°C for long-term storage.

Troubleshooting

Observation	Possible cause	Recommended action
Low or inconsistent yield	Plates stored incorrectly	Store plates in an upright position at room temperature. Examine the plate or row containing the beads before removing the seal for an indication of how to proceed.
		For plates that have been inverted, store them upright for at least 24 hours, then check that the beads form a tight dark pellet in the center of the bottom of the well before unsealing the plate.
		For plates without beads in that were not stored correctly, flick the plates in a fast downward motion to ensure that the materials are in the well and not on the seal before unsealing.
		For plates that were stored inverted, the beads are dry and uneven in the bottom of the wells and or not fully resuspended. Flick the plate in a fast downward motion to remove the fluid from the seal, then gently vortex to resuspend the beads before unsealing. A gentle brief centrifugation can be performed after resuspension but is not always necessary.
	After removing the seal there are some wet or dry beads on the seal	For wet beads, carefully pipet the liquid from the seal back to its proper well.
		For dry beads, resuspend the dry beads in Nuclease-Free water, then carefully pipet the liquid from the seal back to its proper well.
	There are bubbles in the wells	Centrifuge the plates to remove the bubbles before use.
	The wells are blocked	Remove any seal covering well openings and blocking tip comb access. Seal remnants on the plate edge will not interfere with tip comb access and do not have to be removed.
		If the seal has delaminated and left a transparent seal over the well then rotate plate 180 degrees and peel diagonally from the corner that is now on the bottom left. If the problem persists, call technical support.
	Incorrect heat block installed	Install the correct deep well heat block.
	Sample input limits below or above recommend amounts	Consult user guide for recommended sample input ranges.

Labeling symbols

The symbol present on the product label is explained in the following table.



Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

Thermo Fisher Scientific Baltics UAB | V.A. Graiciuno 8, LT-02241 | Vilnius, Lithuania
For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

The information in this guide is subject to change without notice.

DISCLAIMER: TO THE EXTENT ALLOWED BY LAW, THERMO FISHER SCIENTIFIC INC. AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Revision history: Pub. No. MAN0018361

Revision	Date	Description
B.0	24 January 2020	Revising product name from "KingFisher Flex-Ready" to "KingFisher™ Ready".
A.0	15 March 2019	New document

Important Licensing Information: This product may be covered by one or more Limited Use Label Licenses. By use of this product, you accept the terms and conditions of all applicable Limited Use Label Licenses.

©2020 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified.