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KingFisher™ Duo-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 Numbers A36584, A36584SMP

Pub. No. MAN0018362 **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™ Duo-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™ Duo Prime 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™ Duo-Ready DNA Ultra 2.0 Prefilled Plates

Component	Quantity	Storage
Prefilled 96 Deep well plates suitable for 12 gDNA isolations per plate	8	Store upright at 15°C to 25°C
Enhancer Solution	1 bottle	
Proteinase K Solution	1 bottle	
Elution Solution	1 bottle	
12 Pin Deep well Tip Combs	8	
Elution strips	8	
Elution caps	8	
Reusable elution strip holder for filling purposes	1	

If desired, supplementary Elution Solution (Cat. No. A36582) can be purchased separately from **thermofisher.com**.

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™ Duo Prime Magnetic Particle Processor with 96 deep-well head and a 96 deep well heating block (not a standard heating block)	5400110
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
Microfuge mixer capable of shaking tubes at a minimum of 900 rpm	88880023
Materials	
microfuge tubes	MLS
4N6FLOQSwabs™, regular tip	4473979
Reagents	
Nuclease-Free Water	AM9932
Phosphate Buffered Saline (PBS (1X), pH 7.4)	10010023

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.

- 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.

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™ Duo Prime

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	12 Deep well magnetic head
Heat block	12 well heat strips for 96 well plates

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

b. Ensure that the proper program (**DuoReadyDNA_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 an elution strip from the box, then place into the elution strip holder.

b. Use an indelible marker to asymmetrically mark the orientation of the elution strip to prevent sample mix-ups when loading and retrieving the elution strip from the Duo instrument.

c. Gently invert the bottle containing Elution Solution.

   Avoid creating bubbles.

d. Add 100 µL of Elution Solution to the wells of the elution strip, then cap the strip with the caps provided until ready for use.

e. Remove one prefilled plate from the box, then sharply flick the plate downward before removing the seal (for instructions on removing plate seals, see "Before each use of the kit" on page 2).

f. Transfer appropriate amount (according to the following table) of Enhancer Solution first, then sample, and lastly Proteinase K, to each well in Row A in the prefilled 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.

Table 4 Duo-Ready DNA Ultra 2.0 Prefilled Plate (Plate 1) layout

Row ID	Plate Row	Reagent	Volume per well
Sample	A	Sample	Empty
Lysis/Binding (L/B)	B	Lysis/Binding	500 µL L/B Solution
Magnetic beads + Water	C	Magnetic bead solution	40 µL Beads + 160 µL nuclease free H_2O
Wash I	D	Wash I	1,000 µL
Wash II	E	Wash II	1,000 µL
Wash II	F	Wash II	500 µL
Empty	G	Empty	Empty
Tip comb	H	12 pin tip comb	
Duo elution strip			
Elution Solution	—	Elution Solution	100 µL

g. Open a tip comb package with scissors, then gently flex the tip comb until it appears flat before placing the tip comb in Row H of the 96 deep well plate.

h. Start the run on the instrument.

2	Prepare Sample Plate and digest with Proteinase K *(continued)*	i. When prompted by the instrument, place the plate onto the instrument, then secure the uncapped Elution strip on the deck next to the plate with the clamp. Note the orientation of the elution strip when loading into the Duo-Prime instrument. Note: Ensure caps have been removed from the elution strip.
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. Transfer 400 µL of the prefilled Lysis/Binding Solution from Row B to the well directly above in Row A. For example: The contents of well B1 will be transferred to well A1. Using a 12-channel pipette is the best method for easy and quick transfer of row B lysis/binding solution to row A digested sample. Note: There is no need to mix. b. Immediately place the plate back onto 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 Strip from the instrument, then transfer the eluate to the final tube/plate of choice for final storage. Pay careful attention to orientation of the elution strip to avoid sample mix-up. Note: Elution strips are not recommended for short or long term storage of the eluted DNA. 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™ Duo Prime

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 fresh 1.5 ml microfuge tube.

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

b. Break enough of the stick off the swab so that the swab sits in the microfuge tube 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 (12 samples)
Enhancer Solution	40 µL	520 µL
PBS[2]	400 µL	5.2 mL
Proteinase K	40 µL	520 µL
Total volume	**480 µL**	**6.24 mL**

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

IMPORTANT! Prepare immediately before use. The Mix is not stable for prolonged periods and will result in a reduction of DNA yield.

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

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

e. Carefully close all microfuge tubes, then shake at 900 rpm for 5 minutes.

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

IMPORTANT! Arrange tubes or tube rack in the incubator to allow adequate flow around the tubes or tube racks to ensure that samples quickly reach and maintain the incubation temperature.

g. Remove one prefilled plate from the box, then sharply flick the plate downward before removing the seal (for instructions on removing plate seals, see "Before each use of the kit" on page 2).

h. After the proteinase K digestion is complete, transfer 480 µL of lysate to each well in Row B of the prefilled plate.

   Note: There is no need to mix.

i. Confirm that the lysate (420–480 µL) from the buccal swab digestion tubes is transferred to Row B, which contains 500 µL of the Lysis /Binding Solution.

   Note: Row A is left empty for buccal swab purifications.

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	12 Deep well magnetic head
Heat block	12 well heat strips for 96 well plates

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

b. Ensure that the proper program (**DuoReadyDNA_Ultra2_Buccal96**) has been downloaded from the product page and loaded onto the instrument.

3 Prepare Sample Plate and Purify the gDNA

a. Remove an elution strip from the box, then place into the elution strip holder.

b. Use an indelible marker to asymmetrically mark the orientation of the elution strip to prevent sample mix-ups when loading and retrieving the elution strip from the Duo instrument.

c. Gently invert the bottle containing Elution Solution.

Avoid creating bubbles.

d. Add 100 µL of Elution Solution to the wells of the elution strip, then cap the strip with the caps provided until ready for use.

Table 5 Duo-Ready DNA Ultra 2.0 Prefilled Plate (Plate 1) layout

Row ID	Plate Row	Reagent	Volume per well
Sample	A	Sample	Empty
Lysis/Binding (L/B)	B	Lysis/Binding	500 µL L/B Solution
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Wash I	D	Wash I	1,000 µL
Wash II	E	Wash II	1,000 µL
Wash II	F	Wash II	500 µL
Empty	G	Empty	Empty
Tip comb	H	12 pin tip comb	
Duo elution strip			
Elution Solution	—	Elution Solution	100 µL

e. Open a tip comb package with scissors, then gently flex the tip comb until it appears flat before placing the tip comb in Row H of the 96 deep well plate.

f. Start the run on the instrument.

g. When prompted by the instrument, place the plate onto the instrument, then secure the Elution strip on the deck next to the plate with the clamp and press **Start**.

Note the orientation of the elution strip when loading into the Duo-Prime instrument.

Note: Ensure caps have been removed from the elution strip.

h. At the end of the run, immediately remove the Elution Strip from the instrument, then transfer the eluate to the final tube or plate of choice for final storage.

Pay careful attention to orientation to avoid sample mix-up.

Note: Elution strips are not suitable for short or long term storage of the eluted DNA.

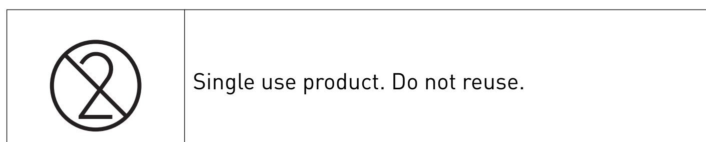
The purified DNA is ready for immediate use. Store eluted DNA at –20°C for long-term storage.

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.



Symbol	Description
(single use symbol)	Single use product. Do not reuse.

Limited product warranty

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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.

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Revision history: Pub. No. MAN0018362

Revision	Date	Description
B.0	15 November 2019	Updated Prepare samples and digest with Proteinase K (page 5) steps h and i, and removed step f.
A.0	15 March 2019	New document

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