

Thermo Scientific KingFisher®

User Manual

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Thermo Scientific
KingFisher[®]
User Manual

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Contents

1	Safety Symbols and Markings	6
2	About the User Manual	7
3	Introduction to the KingFisher	8
4	Functional Description	10
5	Installation	15
6	Routine Operation	19
7	Maintenance	23
8	Technical Specifications	26
9	Troubleshooting Guide	29
10	Warranty Certificate	34
11	Ordering Information	35
12	Glossary and Abbreviations	36
13	Index	38
14	Table of Figures	41
15	Appendix A. Application Protocols for KingFisher	42
16	Appendix B. Thermo Scientific KingFisher Brief User's Guide	45
17	Appendix C. Thermo Scientific KingFisher Feedback Form	46
18	Appendix D. Addresses	47
19	Appendix E. Supplementary Application Protocols	48

Table of Contents

1	Safety Symbols and Markings	6
2	About the User Manual	7
3	Introduction to the KingFisher	8
3.1	Intended use	8
3.2	Principle of operation	8
3.3	Advantages of processing magnetic particles with the KingFisher	9
4	Functional Description	10
4.1	Instrument layout	10
4.1.1	Front view	10
4.1.2	Back view	11
4.1.3	Internal view	11
4.2	KingFisher magnetic particle processor	12
4.3	Principle of the KingFisher magnetic particle processor	13
4.4	Working with a magnetic rod	13
4.4.1	Collecting magnetic particles	13
4.4.2	Releasing magnetic particles	13
4.4.3	Washing magnetic particles	14
4.4.4	Incubation	14
4.4.5	Changing the volume during the magnetic particle processing	14
5	Installation	15
5.1	What to do upon delivery	15
5.1.1	How to unpack	15
5.1.2	Checking delivery for completeness	15
5.1.3	Checking for damage during transport	15
5.1.4	Environmental requirements	16
5.1.5	Things to avoid	16
5.1.6	Technical prerequisites	16
5.2	Setups before you put the instrument into operation	16
5.2.1	How to release the transport lock	16
5.2.2	How to ensure the startup	18
5.3	Operational check	18
6	Routine Operation	19
6.1	Switching on	19
6.2	How to use the keypad and display	19
6.3	How to START	20
6.4	Shutdown	22
7	Maintenance	23
7.1	Regular and preventive maintenance	23
7.1.1	How to clean the plate carrier	23
7.1.2	How to clean the magnetic rods	23
7.1.3	How to clean the processing chamber	23
7.2	Disposal of materials	24
7.3	Decontamination procedure	24
7.4	How to pack for service	25
7.5	Service contracts	25
7.6	Disposal of the instrument	25

8	Technical Specifications	26
8.1	General specifications	26
8.2	Safety specifications	27
8.3	In conformity with the requirements	28
9	Troubleshooting Guide	29
9.1	Error messages and warnings	29
9.2	Troubleshooting guide	30
9.3	Frequently asked questions (FAQ) about the KingFisher	31
9.4	Service request protocol	32
9.4.1	Decontamination	32
9.4.1.1	Certificate of Decontamination	33
10	Warranty Certificate	34
10.1	Warranty limitations	34
11	Ordering Information	35
11.1	List of accessories	35
11.2	List of spare parts	35
11.3	List of recommended spare parts	35
12	Glossary and Abbreviations	36
12.1	Keywords for web pages	36
12.2	Literature	37
13	Index	38
14	Table of Figures	41
15	Appendix A. Application Protocols for KingFisher	42
A.1	Example of a KingFisher genomic DNA purification protocol from blood	42
A.2	Example of a KingFisher total RNA purification protocol	43
16	Appendix B. Thermo Scientific KingFisher Brief User's Guide	45
17	Appendix C. Thermo Scientific KingFisher Feedback Form	46
18	Appendix D. Addresses	47
19	Appendix E. Supplementary Application Protocols	48

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1 Safety Symbols and Markings

These symbols are intended to draw your attention to essential information and alert you to the presence of hazards as indicated.

SAFETY SYMBOLS USED IN THE KINGFISHER



Power ON



Power OFF



WEEE symbol This product is required to comply with the European Union's Waste Electrical & Electronic Equipment (WEEE) Directive 2002/96/EC.

WARNING MARKINGS USED IN THE DOCUMENTATION



Caution: risk of electric shock.



Caution: biohazard risk.



Caution: risk of personal injury to the operator or a safety hazard to the surrounding area.



Caution: risk of damage to the instrument, other equipment or loss of performance or function in a specific application.

2 About the User Manual

This user manual has been written for the actual end user (for example, laboratory technician) and provides information on the Thermo Scientific KingFisher magnetic particle processor, including installation and operating instructions.

Read the manual in its entirety prior to operating the instrument.

This user manual has been designed to give you the information you need to:

- Install the KingFisher
- Use the KingFisher in routine – the processing step
- Perform basic maintenance procedures.

This user manual also describes all the features and specifications of the KingFisher hardware and on-board software.

Chapter 15 Appendix A. Application Protocols for KingFisher explains the processing principles and procedures.

In Chapter 9 Troubleshooting Guide you will find explanations of all error messages and a problem-solving guide. The user should be familiar with the contents of Chapter 7 on maintenance.

For warranty and ordering information, refer to Chapters 10 Warranty Certificate and 11 Ordering Information.

In an effort to produce useful and appropriate documentation, we appreciate your comments on this document to your local Thermo Fisher Scientific representative.

3 Introduction to the KingFisher

3.1 Intended use

The KingFisher magnetic particle processor (*Fig. 3.1*) is intended for professional research use by trained personnel. The instrument is intended for automated transfer and processing of magnetic particles in a microwell scale. Use for self-testing is excluded. It is recommended that Good laboratory Practices (GLP) are followed to guarantee reliable analyses.

3.2 Principle of operation

The KingFisher magnetic particle processor (*Fig. 3.1*) is designed for the automated transfer and processing of magnetic particles in a microwell scale.

The principle of the KingFisher system (patents pending) is based on the use of magnetic rods covered with the disposable, specially designed tip combs and microstrips. These are provided with the reagent kit. The instrument functions without any sucking or aspiration parts or devices.

Samples and reagents including magnetic particles are dispensed into the wells according to the corresponding kit instructions. The use of microplate format allows the use of microwell-scale volumes and existing liquid-handling tools. The steps of the protocol that are selected by the user via the keypad and display are preloaded in the on-board software.



Fig. 3.1 KingFisher magnetic particle processor

3.3 Advantages of processing magnetic particles with the KingFisher

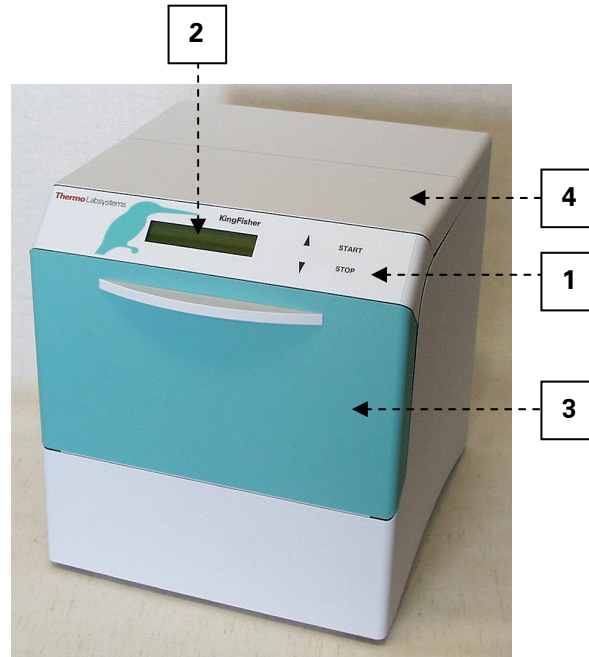
The KingFisher magnetic particle processor provides several advantages relating mainly to the principle of operation in that it:

- Enables automation of complicated manual steps
- Enables simultaneous processing and purification, for example, of double-stranded DNA
- Enables a quicker reaction and a more efficient wash due to the simultaneous shaking during incubation and washing
- Facilitates a good collection of bead-bound sample due to the efficiency of the magnet
- Prevents carry-over due to the fact that the same tip is only used for one sample and furthermore that the reagents have been pipetted into their own wells (different reagents are not added into the same well)
- Is easy to carry out the whole processing with the aid of an internal program.

4 Functional Description

4.1 Instrument layout

4.1.1 Front view



- 1 Keypad
- 2 Display
- 3 Front lid
- 4 Top lid

Fig. 4.1 KingFisher front view with lids closed

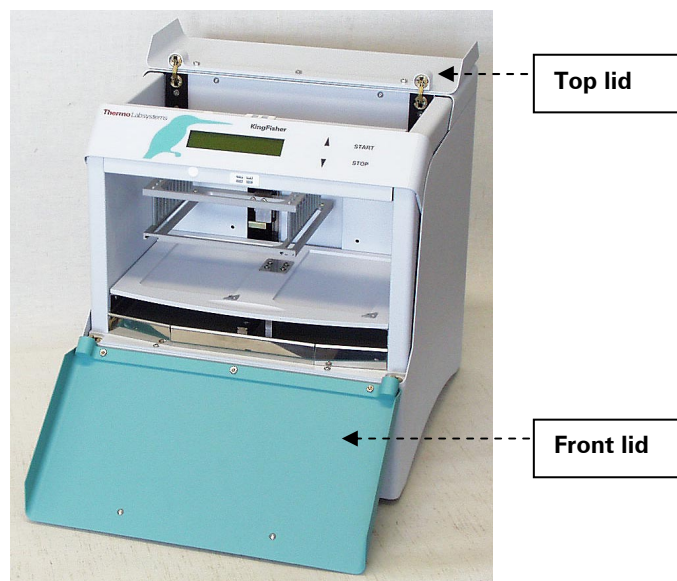
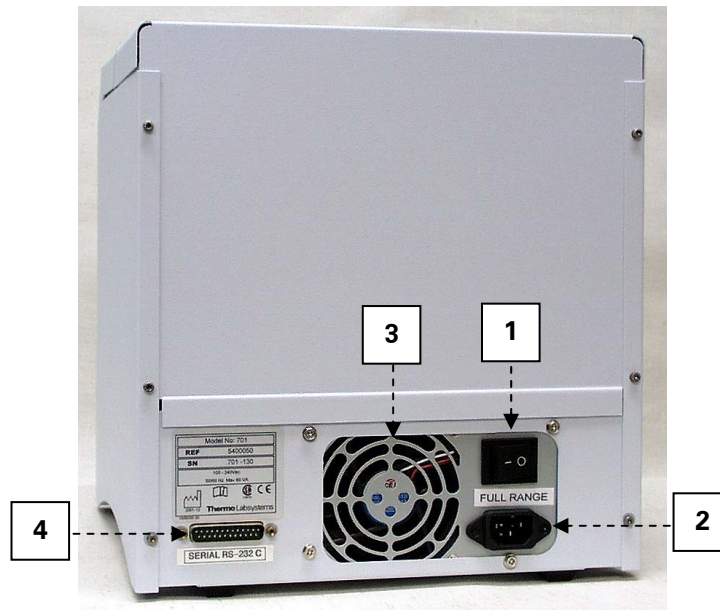


Fig. 4.2 KingFisher front view with lids open

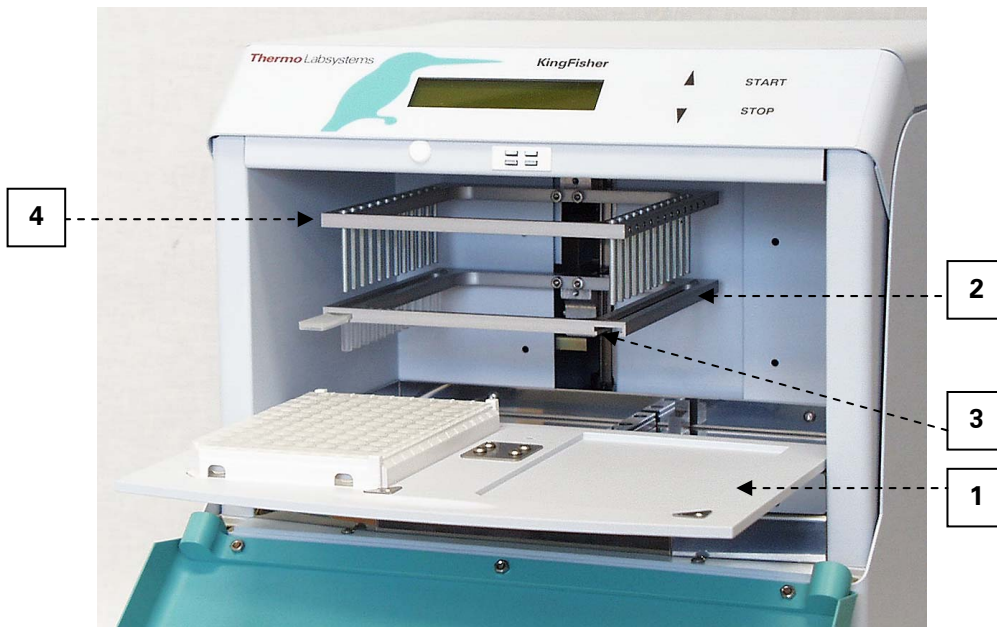
4.1.2 Back view



- 1 ON/OFF switch
- 2 Mains power supply socket
- 3 Cooling-air outlet
- 4 Serial port RS-232C

Fig. 4.3 KingFisher rear view

4.1.3 Internal view



- 1 Plate carrier
- 2 Tip comb holder
- 3 Tip comb slot
- 4 Fixed magnetic rod holder

Fig. 4.4 KingFisher internal view

4.2 KingFisher magnetic particle processor

The KingFisher magnetic particle processor (*Fig. 4.5*) is designed for maximum two 96-well plates and special microstrips, which are compatible with the tip comb. The plate(s) is (are) kept stationary and the only moving assembly is the processing head with tip combs and magnetic rods. The head consists of two vertically moving platforms. One is needed for the magnetic rods (2 x 12 pieces) and the other one for the plastic tip combs (*Fig. 4.4*).

One plate contains twelve separate microstrips and one sample processing uses one microstrip containing eight wells. One tip comb containing twelve tips is used for processing twelve samples at a time within one plate.

Before starting the magnetic particle processing via the keypad and display, the samples and reagents are dispensed into the wells and the tip comb(s) is (are) loaded into its (their) slot(s). The plate(s) is (are) placed onto the moving plate carrier in the correct position and the plate carrier is pushed into the end position. During the operation, the front and top lids can be closed (*Fig. 4.1*) or open (*Fig. 4.2*). Closed lids protect the processing against environmental contamination.

The operating principle employed is MPP (*inverse magnetic particle processing*) technology (*Fig. 4.6*). Rather than moving the liquids, the magnetic particles are moved from microwell to microwell containing specific reagents, in contrast to the external magnet method. Magnetic particles are transferred with the aid of magnetic rods covered with disposable, specially designed plastic tip combs.

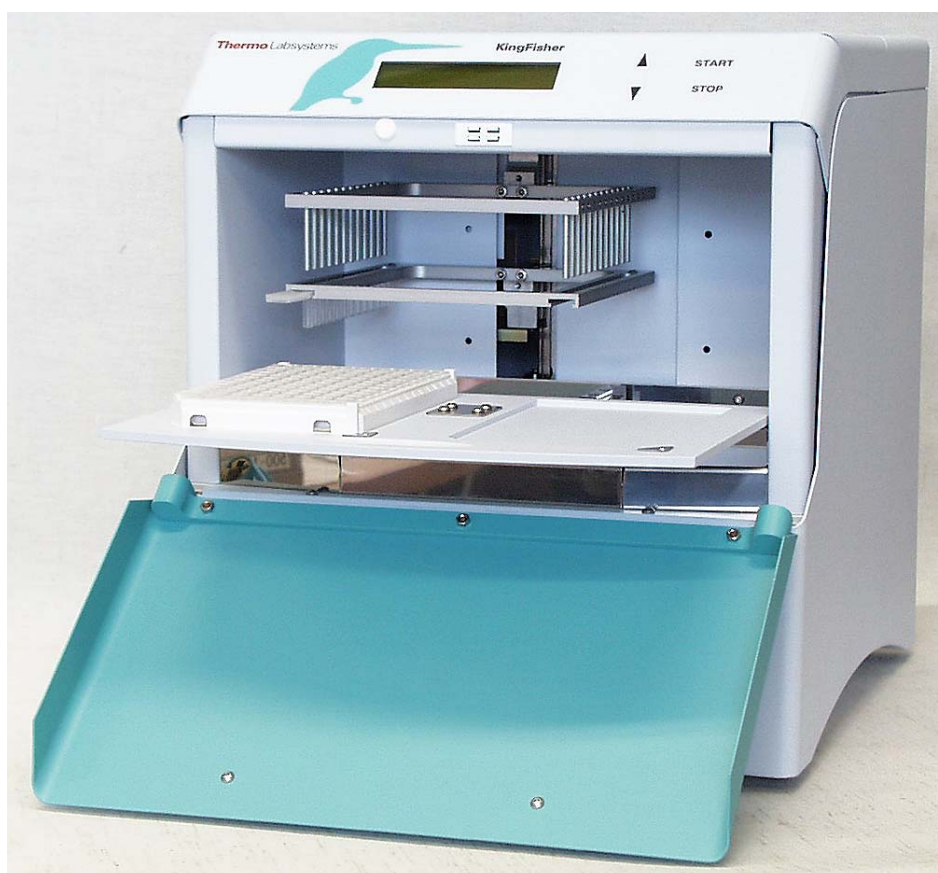


Fig. 4.5 KingFisher magnetic particle processor

4.3 Principle of the KingFisher magnetic particle processor

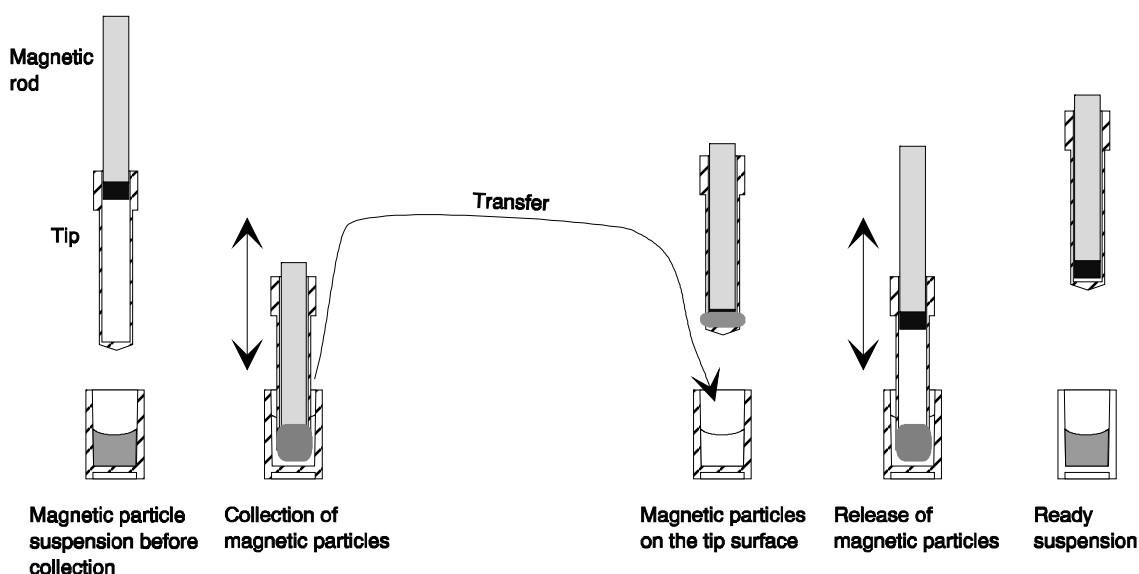


Fig. 4.6 Inverse magnetic particle processing

4.4 Working with a magnetic rod

Working with magnetic particles can be divided into five separate processes:

- collecting particles
- releasing particles
- washing particles
- incubation
- concentration

4.4.1 Collecting magnetic particles

During the collection of the magnetic particles, the magnetic rod (bar) is fully inside the tip. The magnetic rods together with the tip comb(s) move slowly up and down in the microwells and the magnetic particles are collected onto the wall of the tips. The magnetic rods together with the tip comb(s), having collected the magnetic particles, can be lifted out of the microwells and transferred into the next microwells.

4.4.2 Releasing magnetic particles

After collection of the magnetic particles, the magnetic rods together with the tip comb(s) are lifted from the microwells, the magnetic rods are lifted off and the tip comb(s) is (are) lowered into the next microwells containing a reagent.

Magnetic particles are released by moving the tip comb(s) up and down several times with considerably high speed until all the particles have been mixed with the substance in the next reaction.

4.4.3 Washing magnetic particles

Washing the magnetic particles is a frequent and an important processing phase. Washing is a combination of the release and collection processes in a microwell filled with washing solution.

To maximize washing efficiency, the magnetic rods together with the tip comb(s) are designed to have minimized liquid-carrying properties.

4.4.4 Incubation

To keep the magnetic particle suspension evenly mixed in long-running reactions, the tip comb(s) can be moved up and down from time to time.

4.4.5 Changing the volume during the magnetic particle processing

The volume of the first microwell can be larger than the volume of the next microwell and this is used for *concentration* purposes (see Fig. 4.7 below).

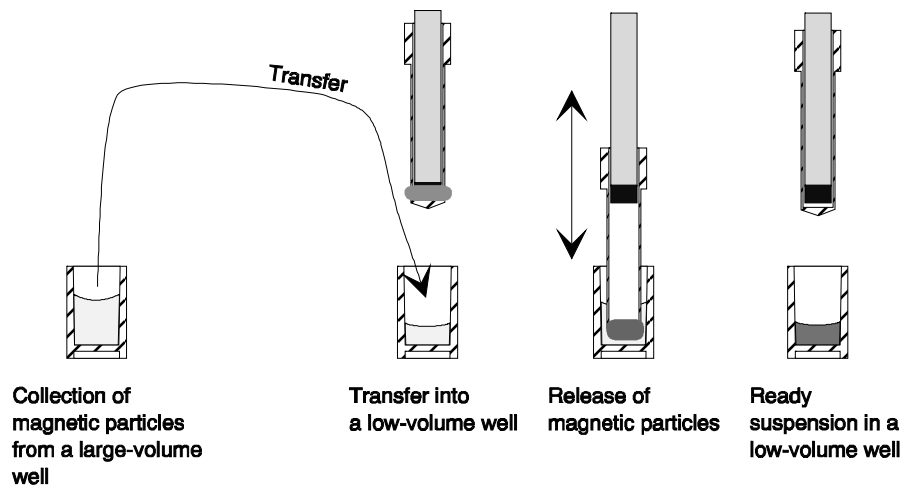


Fig. 4.7 Concentration step during magnetic particle processing

5 Installation

5.1 What to do upon delivery

5.1.1 How to unpack

Move the unpacked instrument to its site of operation. Unpack the KingFisher instrument and accessories carefully with the arrows on the transport package pointing upwards. The following notes and instructions are sent with the instrument and are immediately available when you open the package:

- packing instructions/packing list
- Thermo Scientific KingFisher Feedback Form
- Warranty Certificate card
- *KingFisher User Manual*.



Caution: Do not touch or loosen any screws or parts other than those specially designated in the instructions. Doing so might cause misalignment and will void the instrument warranty.

To lift the instrument, put your fingers under the bottom on either sides and lift it with your back straight.

Retain the original packaging for future transportation. The packaging is designed to assure safe transport and minimize transit damage. Use of alternative packaging materials may invalidate the warranty. Also retain all instrument-related documentation provided by the manufacturer for future use.

5.1.2 Checking delivery for completeness

Check the enclosed packing list against order. If any parts are missing, contact your local Thermo Fisher Scientific representative or Thermo Fisher Scientific Oy.

5.1.3 Checking for damage during transport

Visually inspect the transport package, the instrument and the accessories for any possible transport damage.

If the carton has been damaged in transit, it is particularly important that you retain it for inspection by the carrier in case there has also been damage to the instrument.

Visually check all interconnections in the basic instrument. Check that there are no loose parts inside the instrument.

If any parts are damaged, contact your local Thermo Fisher Scientific representative or Thermo Fisher Scientific Oy.

5.1.4 Environmental requirements

When you set up your KingFisher, avoid sites of operation with excess dust, vibrations, strong magnetic fields, direct sunlight, draft, excessive moisture or large temperature fluctuations.

- Make sure the working area is flat, dry, clean and vibration-proof and leave additional room for cables, lids, etc.
- Make sure the ambient air is clean and free of corrosive vapors, smoke and dust.
- Make sure the ambient temperature range is between +10°C (50°F) and +40°C (104°F).
- Make sure relative humidity is between 10% and 90% (non-condensing).

Leave sufficient space (at least 10 cm) at both sides and at the back of the unit to allow adequate air circulation.

The KingFisher does not produce operating noise at a level that would be harmful. No sound level measurements are required after installation.



Warning: Do not operate the instrument in an environment where potentially damaging liquids or gases are present.

5.1.5 Things to avoid

Do not smoke, eat or drink while using the KingFisher. Wash your hands thoroughly after handling test fluids. Observe normal laboratory procedures for handling potentially dangerous samples. Use proper protective clothing. Use disposable gloves. Be sure the working area is well-ventilated.

Never spill fluids in or on the equipment.



Warning: This product contains strong permanent magnets. People wearing a pacemaker or metallic prostheses should not use this product. A pacemaker or prostheses may be affected or damaged if it comes in very close contact with a strong magnetic field.

5.1.6 Technical prerequisites

Place the instrument on a normal laboratory bench. The net weight of the unit is approx. 10 kg (23 lbs.).

The instrument operates at voltages of 100 – 240 Vac and a frequency range of 50/60 Hz.

5.2 Setups before you put the instrument into operation

5.2.1 How to release the transport lock

Note: THERE IS ONE TRANSPORT LOCK PRESENT (*Fig. 5.2*). A white tag with the red text 'CAUTION! Remove Transport Lock Before Use' is fastened to the instrument to attract the user's attention (*Fig. 5.1*). The tag in *Fig. 5.1* and the black arrows in *Fig. 5.2* indicate where the transport lock piece is situated. Remove the tag and release the transport lock. Ensure that the transport lock has been released before you put the instrument into operation.

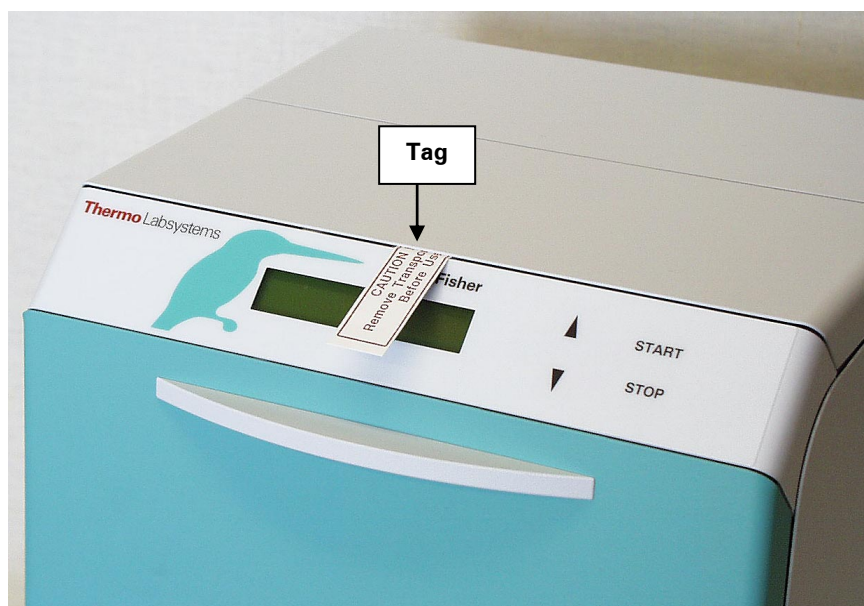


Fig. 5.1 KingFisher transport lock tag present before release of the lock piece

1. To remove the transport lock, unscrew the three screws (Fig. 5.2) with the 2.5 mm hexagonal screwdriver supplied.
2. Remove the transport lock piece.
3. Save the transport lock piece and screws and the transport lock warning tag for future transportation.

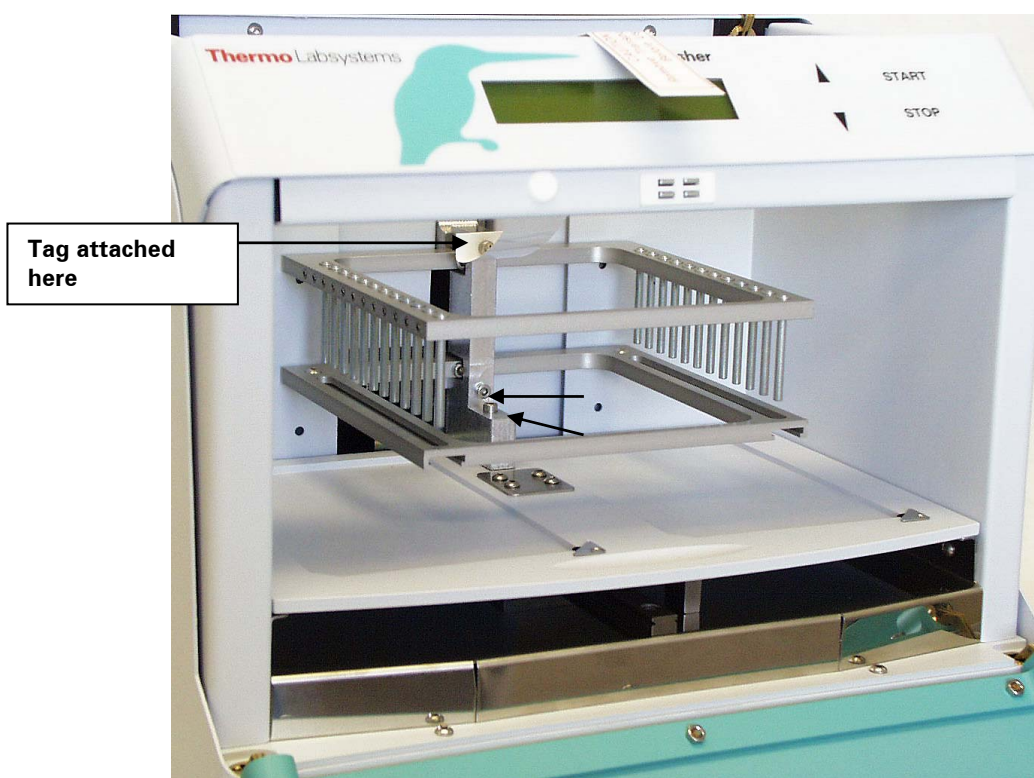


Fig. 5.2 Location of the transport lock screws

5.2.2 How to ensure the startup



1. **Warning:** Ensure that the mains switch (*Fig. 4.3*) on the bottom left of the back panel is in the OFF position.



2. Connect the mains supply cable to the *mains power socket* (*Fig. 5.3*) at the bottom left of the back panel. If any other type of mains supply cable than supplied is needed, use only cables certified by the local authorities. Before you plug in the power cable, ensure that the voltage on the rating label at the bottom left of the back panel (*Fig. 4.3*) corresponds to the local voltage.
3. Connect the instrument to a correctly installed line power outlet, which has a protective conductor that is grounded.



Warning: Never operate your instrument from a power outlet that has no ground connection.



Fig. 5.3 Connecting the mains supply cable

5.3 Operational check

1. First switch the instrument ON. The instrument performs initialization tests and adjustments. The initialization tests are so-called self-tests.
2. The display quickly shows the internal software version and after that the display shows one of the protocols in the internal memory. This happens when the initialization tests and adjustments have been completed.
3. We recommend that you carry out an empty run (for example, a demonstration) to verify proper instrument operation.

6 Routine Operation

6.1 Switching on

Before you switch on the KingFisher, ensure that the voltage on the rating label at the bottom left of the back panel (*Fig. 4.3*) corresponds to the local voltage.

6.2 How to use the keypad and display

The liquid crystal display is a 20 character by 2 row (line) LCD.

The lower line of the display can show the steps of the protocol, while the processing is running. Furthermore, if errors occur they will be displayed on the lower row.

The keypad and display are shown in *Fig. 6.1*.



Fig. 6.1 KingFisher keypad

The keys available are:

- *two cursor keys (or arrow keys, direction keys)*: to select the processing step to be run

- ▲ To change to the previous/next protocol by scrolling up.
- ▼ To change to the next protocol by scrolling down.

- *two function keys*

START To initiate the processing step.

STOP To pause/terminate the processing step. In short:
 STOP (paused)/START (the instrument continues after a Pause step)
 STOP (paused)/STOP (the processing is terminated).

6.3 How to START

- First of all slide the plate carrier out (*Fig. 6.2*).

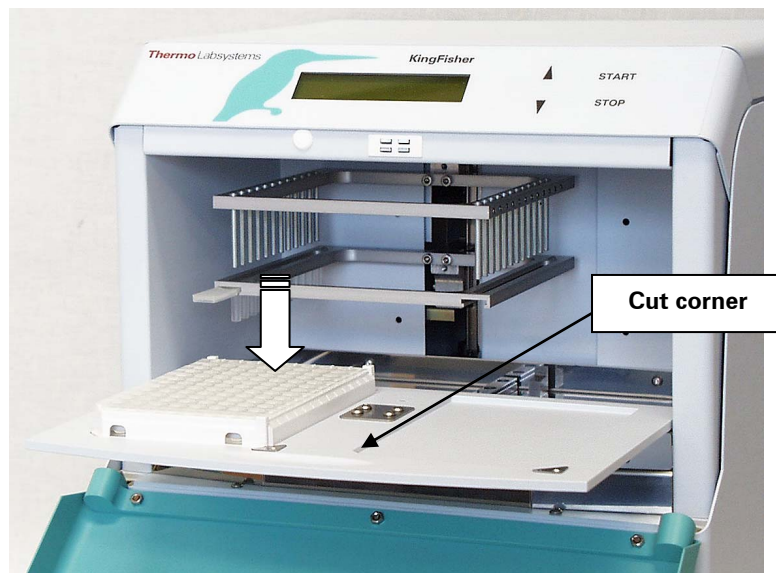


Fig. 6.2 Loading the plate carrier

- Load only maximum two special plates (*Fig. 6.5*) onto the plate carrier for processing at a time. The plates have to be positioned so that the cut corners face left down (*Fig. 6.2*). They also have to be in their places. Slide the plate carrier fully back in.

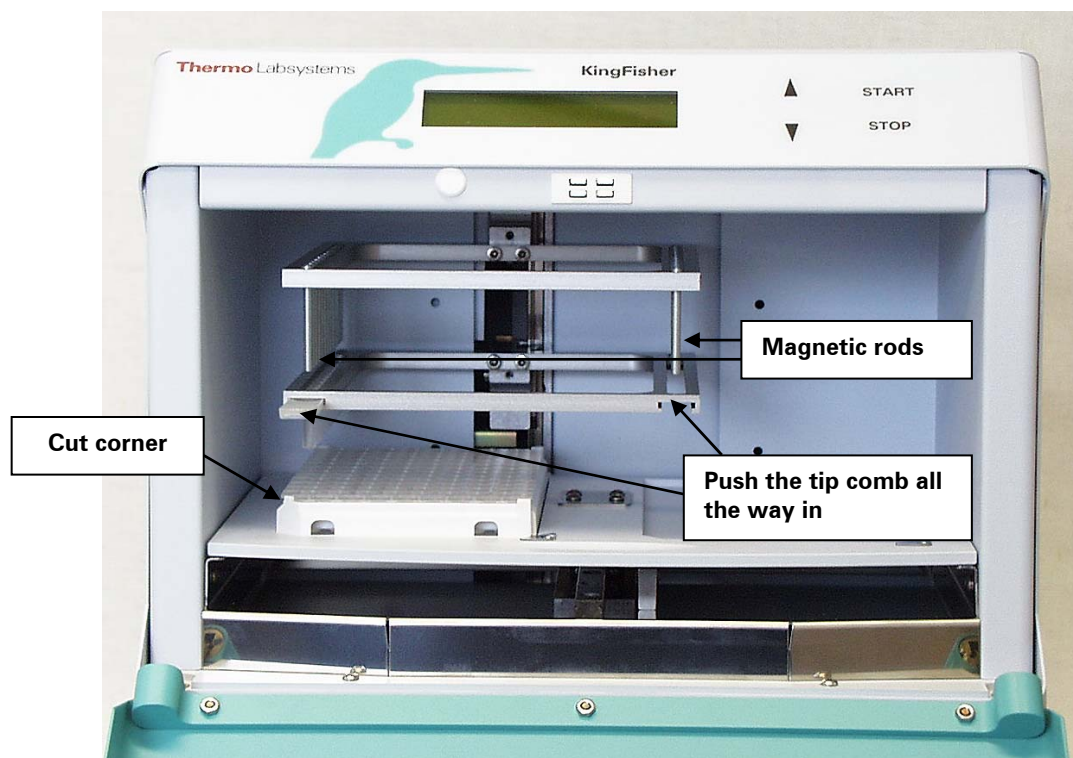


Fig. 6.3 Positioning of the plate(s) and insertion of the tip comb(s)

- The tip comb(s) always has (have) to be in its (their) slot(s) and fully inserted (*Fig. 6.3*). The instrument also functions with either one microplate or one to twelve microstrips depending on the amount of samples. At least one tip comb (*Fig. 6.4*) always has to be inserted into either of the narrow slots meant for the tip comb.

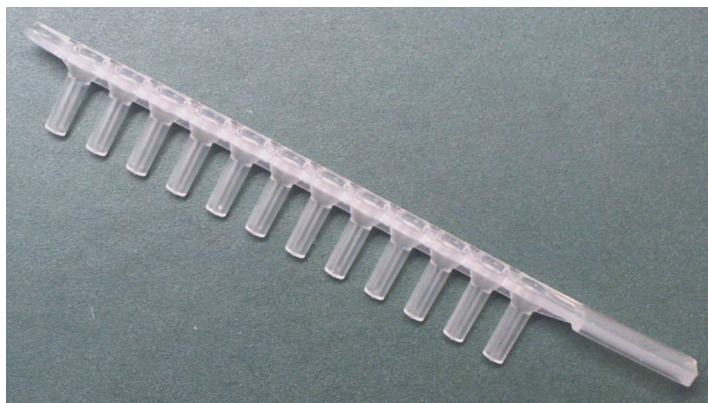


Fig. 6.4 Tip comb specially designed for the KingFisher

- Close the front lid if you want to. The front lid protects the instrument against environmental contamination.

Note: The front lid and/or top lid can be left open if desired. This action does not break the run.

- Select the name of the method, for example, **Genomic_DNA_1**.
- Use the **START** key to initiate the run.

For startup refer to Section 6.1 Switching on.

Note: The samples are collected immediately after the run and transferred into other tubes for longer storage.

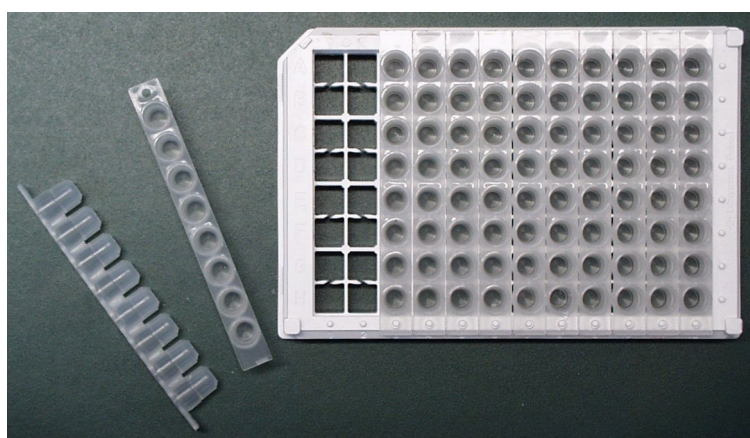


Fig. 6.5 Plate containing microstrips specially designed for the KingFisher

6.4 Shutdown

Switch the KingFisher off by pressing the power switch (*Fig. 4.3*) at the bottom left of the back panel of the instrument into the OFF position.



Remove any plate still on the plate carrier. Dispose of all microplates as biohazardous waste.



Remove any tip combs still in the instrument. Dispose of tip combs as biohazardous waste.

Wipe the plate carrier surface and the neighboring instrument surface with a soft cloth or tissue paper moistened with distilled water, a mild detergent (SDS, sodium dodecyl sulfate) or soap solution.

If you have spilt infectious agents on the plate carrier, disinfect with 70% alcohol or some other disinfectant (see Section 7.3 Decontamination procedure).

7 Maintenance

7.1 Regular and preventive maintenance

For reliable daily operation keep the instrument free of dust and liquid spills.

Abrasive cleaning agents are not recommended, because they are likely to damage the paint finish.

We recommend you clean the case of the instrument periodically to maintain its good appearance. A soft cloth dampened in a warm, mild detergent solution will be sufficient.

Clean the outside of the instrument and the plate carrier with clean low-pressure compressed air or a cloth dampened with water or a mild detergent when necessary.

Although the KingFisher is constructed from high-quality materials, you must immediately wipe away spilt saline solutions, solvents, acids or alkaline solutions from outer surfaces to prevent damage.



Painted surfaces can be cleaned with most laboratory detergents. Dilute the cleaning agent as recommended by the manufacturer. Do not expose painted surfaces to concentrated acids or alcohols for prolonged periods of time as damage may occur.

Clean the display areas with a mild laboratory detergent. The keypad has a wipe-clean surface.

Plastic covers and surfaces can be cleaned with a mild laboratory detergent or alcohol.



If any surfaces have been contaminated with biohazardous material, a mild sterilizing solution should be used.



Do not autoclave any part of this instrument.

7.1.1 How to clean the plate carrier

Keep the plate carrier surface clean to avoid dust and dirt entering into the instrument. Clean the plate carrier surface at least once a week using a soft cloth or tissue paper soaked in a mild detergent solution (SDS), soap solution or alcohol.

If you have spilt infectious agents on the plate carrier, disinfect according to Section 7.3 Decontamination procedure.

7.1.2 How to clean the magnetic rods

If required, clean the magnetic rods using a soft cloth or tissue paper soaked in a mild detergent solution (SDS), soap solution or alcohol.

7.1.3 How to clean the processing chamber

If required, clean the processing chamber using a soft cloth or tissue paper soaked in a mild detergent solution (SDS), soap solution or alcohol.

7.2 Disposal of materials

Refer to local regulations for the disposal of infectious material.



The samples can be potentially infectious. Dispose of all used microplates, disposable gloves, syringes, disposable tips, etc. as biohazardous waste.

7.3 Decontamination procedure

If there is any risk of contamination with biohazardous material, the procedure recommended below or some other corresponding decontamination procedure must be performed.

We strongly recommend that the complete decontamination procedure is performed before relocating the instrument from one laboratory to another.

Decontamination is not required for the proper functioning of the instrument.

Example of decontaminants:

- Ethanol 70%
- Virkon solution 1 – 3%
- Glutaraldehyde solution 4%
- Chloramine T
- Microcide SQ 1:64



Always use disposable gloves and protective clothing and operate in a well-ventilated area.

1. Prepare the decontaminant: for example, 200 ml 4% glutaraldehyde solution (or another agent recommended by your safety officer).
2. Empty the plate carrier. Ensure that you are wearing disposable gloves.
3. Switch OFF the power and disconnect the mains supply cable (*Fig. 5.3*).
4. Disinfect the outside of the instrument using a cloth dampened with 70% ethanol.
5. Place the instrument in a large plastic bag. Ensure that the lids are open.
6. Place a cloth soaked in the prepared solution into the bag. Ensure that the cloth does not make contact with the instrument.
7. Close the bag firmly and leave the instrument in the bag for at least 24 hours.
8. Remove the instrument from the bag.
9. Clean the instrument using a mild detergent.
10. Remove any stains using 70% ethanol.

11. After performing this decontamination procedure, enclose a signed and dated Certificate of Decontamination both inside the transport package and attached to the outside of the package.

7.4 How to pack for service

When you ship the instrument for service, remember to:

- Inform about the use of hazardous materials.
- Decontaminate the instrument beforehand.
- Install the transport lock.
- Pack the instrument according to the enclosed packing instructions.
- Use the original packaging to ensure that no damage will occur to the instrument during shipping. Any damage will incur additional labor charges.
- Enclose a dated and signed Certificate of Decontamination (see Section 9.4.1.1) both inside and attached to the outside of the package, in which you return your instrument (or other items).
- Enclose the return authorization number (RGA) given by the Thermo Fisher Scientific representative.
- Indicate the fault after you have been in touch with your local Thermo Fisher Scientific representative or Thermo Fisher Scientific Oy.

See Section 8.1 for details on storage and transportation temperatures.

7.5 Service contracts

We recommend you maintain and service the instrument regularly every twelve months on a contract basis by the manufacturer's trained service engineers. This will ensure that the product is properly maintained and gives trouble-free service. Contact the Thermo Fisher Scientific technical service department for more details.

7.6 Disposal of the instrument



- Decontaminate the instrument prior to disposal. See Sections 7.3, 9.4.1 and 9.4.1.1 on decontamination.
- Dispose of the instrument according to the legislation stipulated by the local authorities concerning take-back of electronic equipment and waste. The proposals for the procedures vary by country.



Thermo Fisher Scientific has contracted with one or more recycling/disposal companies in each EU Member State (European Country), and this product should be disposed of or recycled through them. Further information on Thermo Fisher Scientific's compliance with these Directives, the recyclers in your country, and information on Thermo Scientific products which may assist the detection of substances subject to the RoHS Directive are available at www.thermo.com/WEEERoHS.

- Regarding the original packaging and packing materials, use the recycling operators known to you.
- For more information, contact your local Thermo Fisher Scientific representative.

8 Technical Specifications

8.1 General specifications

Technical specifications

Overall dimensions	W x D x H: 290 x 290 x 310 mm (11.4 x 11.4 x 12.2 in.)
Weight	ca. 10 kg (23 lbs.)
Operating conditions (indoor use)	+10°C – +40°C, RH: 90% max.
Transportation conditions	-40°C – +70°C, packed in transport packaging
Storage conditions	-25°C – +50°C, packed in transport packaging
Mains power supply	100 – 240 Vac, 50/60 Hz, nominal Automatic voltage detection
Power consumption	80 VA max., 50 VA standby
Heat dissipation	273 BTU max.
Computer interface	Serial RS-232C port. Baud rate 9600. Character format 1 start bit, 8 data bits, 1 stop bit, no parity. Flow control XON/XOFF.

Performance specifications

Microplate type (disposable)	Special design, 8 x 12 grid format
Magnetic rods (fixed)	2 x 12 format
Capacity	24 samples/run (2 x 12)
Vessel volumes	20 – 200 µl
Vessel type (disposable)	Special microstrip, 1 x 8 wells
Tip comb (disposable)	Special design, 1 x 12 format
Magnetic particle size	ca. > 1 µm
Collecting efficiency	99%/vessel (washing buffer)
Incubating temperature	Ambient temperature (i.e., no incubation)
Keyboard / Display	START/STOP/two cursor keys/LCD
Software	1 – 8 fixed internal protocols (loading through the external PC). Thermo Scientific KingFisher Software can be used to create and modify protocols.

8.2 Safety specifications

The KingFisher fulfills the following requirements:

EN 61010-1:1993 + A2:1995/IEC 61010-1:1990 + A1:1992 + A2:1995
including CENELEC Common Modifications, US and CA National differences
-Installation Category (Overvoltage Category) II; Pollution Degree 2
EN 61010-1:2001 (Ed. 2)

The safety specifications are also met under the following environmental conditions in addition or in excess to those stated in the operating conditions:

Altitude:	Up to 2000 m
Temperature:	+5°C – +40°C
Mains supply fluctuations:	± 10% from nominal
Installation category (overvoltage category):	II according to IEC 60664-1 (see Note 1)
Pollution degree:	2 according to IEC 60664-1 (see Note 2)

Notes

1. The *installation category* (overvoltage category) defines the level of transient overvoltage, which the instrument is designed to withstand safely. It depends on the nature of the electricity supply and its overvoltage protection means. For example, in CAT II, which is the category used for instruments in installations supplied from a supply comparable to public mains, such as hospital and research laboratories and most industrial laboratories, the expected transient overvoltage is 2500 V for a 230 V supply and 1500 V for a 120 V supply.
2. The *pollution degree* describes the amount of conductive pollution present in the operating environment. Pollution degree 2 assumes that normally only nonconductive pollution, such as dust, occurs with the exception of occasional conductivity caused by condensation.

Both of these affect the dimensioning of the electrical insulation within the instrument.

8.3 In conformity with the requirements

The KingFisher bears the following markings:

Type 700
100 – 240 Vac 50/60 Hz, 80 VA
CE mark
CSA monogram

The KingFisher conforms to the following requirements:

2006/95/EC (Low Voltage Directive)
2004/108/EC (Electromagnetic Compatibility Directive, EMC)
2002/96/EC (Waste of Electrical and Electronic Equipment)

Safety performance:

EN 61010-1:1993 + A2:1995/IEC 61010-1:1990 + A1:1992 + A2:1995
including CENELEC Common Modifications, US and CA National differences
-Installation Category (Overvoltage Category) II; Pollution Degree 2
EN 61010-1:2001 (Ed. 2)

EMC performance:

EN 50081-1:1992	Generic emission standard. Residential, commercial and light industry.
EN 50082-1:1997	Generic immunity standard. Residential, commercial and light industry.
EN 61326-1:1997 + A1:1998 + A2:2001 + A3:2003	Product family standard.

Test standards

Performance limits

EN 55022:1998	Class B, 150 kHz – 1 GHz
EN 61000-3-2:1995 + A1:1998 + A2:1998 + A13:1997 + A14:2000 EN 61000-3-3:1995	Class A
EN 61000-4-2:1995 + A1:1998	4 kV CD, 8 kV AD, Criteria B
EN 61000-4-3:1996 + A1:1998	3 V/m, 80 MHz – 2 GHz, Criteria A
EN 61000-4-4:1995	1 kV, Criteria B
EN 61000-4-5:1995	2 kV line to ground, 1 kV line to line, Criteria B
EN 61000-4-6:1996	3 V _{rms} , 150 kHz – 80 MHz, Criteria A
EN 61000-4-11:1994	30%/10 ms, Criteria B 60%/100 ms, Criteria C 100%/20 ms, Criteria B > 95%/5 s, Criteria C

9 Troubleshooting Guide

9.1 Error messages and warnings

<i>Error message</i>	<i>Warning</i>
Command error Cannot move track Head position error Head timeout Track position error Track timeout Magnets pos. error Magnets timeout Serial number error Serialno already set Too high pos. offset Invalid parameter Out of memory No acceleration tbl NV parameters lost Progmem full	Time already expired

9.2 Troubleshooting guide

Error	Cause	Action
<ul style="list-style-type: none"> • Cannot move track 	The track cannot be moved because the head is not up.	Press STOP . If the fault cannot be remedied, then switch the power on and off.
<ul style="list-style-type: none"> • Head position error 	The calculated head position does not agree with the head home sensor signal.	Check that there is nothing preventing the head from moving up and down.
<ul style="list-style-type: none"> • Head timeout 	The head has not arrived at the home sensor within the allowed time.	Check that there is nothing preventing the head from moving up and down.
<ul style="list-style-type: none"> • Track position error 	The calculated track position does not agree with the track home sensor signal.	Check that there is nothing preventing the track from moving left or right.
<ul style="list-style-type: none"> • Track timeout 	The track has not arrived at the home sensor within the allowed time.	Check that there is nothing preventing the track from moving left or right.
<ul style="list-style-type: none"> • Magnets position error 	The calculated magnetic rod position does not agree with the rod home sensor signal.	Check that there is nothing preventing the magnetic rods from moving up and down. Ensure that the tip comb is fully in.
<ul style="list-style-type: none"> • Magnets timeout 	The magnetic rods have not arrived at the home sensor within the allowed time.	Check that there is nothing preventing the magnetic rods from moving up and down.
<ul style="list-style-type: none"> • Nonvolatile parameters lost 	The nonvolatile parameters of the instrument are lost.	Contact authorized service.
<ul style="list-style-type: none"> • Clattering noise 	Plate(s) and/or microstrip(s) is (are) not in place.	Position the plate(s) correctly. Push the microstrip(s) fully into the frame.
<ul style="list-style-type: none"> • Last release poor 	Plate(s) and/or microstrip(s) is (are) not in place.	Position the plate(s) correctly. Push the microstrip(s) fully into the frame.

9.3 Frequently asked questions (FAQ) about the KingFisher

Q1: What does the KingFisher system do?

A1: Thermo Fisher Scientific now offers a complete KingFisher system – the magnetic particle processor – for the purification and processing of DNA, RNA, proteins and cells. The processor handles particles automatically according to the preloaded purification protocols. For more information on these applications, contact Thermo Fisher Scientific Oy.

Q2: Do I need a PC to control the processor?

A2: Normally the protocol(s) is/are preloaded by Thermo Fisher Scientific and no PC is needed for routine use. KingFisher Software can be used to create new protocols or modify existing protocols if needed. PC-modified protocols are then downloaded into the processor for routine use.

Q3: Will the magnets get weaker? If so, how long can they be used? Can they be replaced?

A3: The KingFisher magnets are made of material that is very stable. The magnetic field will not get weaker.

Magnets can be replaced if needed. Usually there is no need to replace them, but it can be done, for example, if they are mechanically damaged during runs (for example, the plate is not correctly positioned during the run). Changing the magnets can be carried out by local technical service personnel.

Q4: How strong are the magnets? Can they disturb, for example, some sensitive equipment?

A4: The magnetic field is situated just around the magnetic tips, so it is very local. Therefore, the magnetic field should not cause any problems with surrounding equipment.

However, if you touch your floppy disk with the magnetic rod, you will lose all your data. Generally, a normal PC screen may also detect the magnetic field with a minor distortion of the image.

Q5: What happens if I forget to insert the tip combs into the tip comb holder and the magnetic particles stick to the magnetic rods?

A5: Wipe the magnetic rods with a soft cloth or tissue paper soaked in a mild detergent solution, soap solution or alcohol.

Q6: What if magnetic particles remained in the sample well?

A6: If the starting material is too viscous, the magnetic rods will not be able to collect the particles. Dilute the sample and check that the sample is properly homogenized/lysed.

Q7: What if magnetic particles are attached to the tip combs?

A7: This happens sometimes but it will not affect the yield because the sample has been released from the particles.

Q8: Are the volumes of reagents in each well critical?

A8: We strongly recommend that you keep the given volumes within certain limits to avoid failure in performance of the chemical reactions and the processor.

9.4 Service request protocol

If the KingFisher requires service, contact your local Thermo Fisher Scientific representative or Thermo Fisher Scientific Oy. Do not under any circumstances send the instrument for service without any prior contact. It is imperative to indicate the fault and nature of the required service. This will ensure a faster return of the instrument to the customer.

Your local Thermo Fisher Scientific representative or distributor will take care of sending a complaint form (that is, the Warranty Claim Technical Sheet) to the Thermo Fisher Scientific technical service department. The Warranty Claim Technical Sheet contains a more detailed description of the fault, symptom or condition. Give all the necessary information to the distributor, who will fill out and forward the Warranty Claim Technical Sheet to the Thermo Fisher Scientific technical service department.

Check Section 7.4 How to pack for service. You will find instructions on how to proceed before shipping the instrument for service to Thermo Fisher Scientific Oy.

Check that any necessary decontamination procedure has been carried out before packing. See Sections 9.4.1 and 9.4.1.1 on decontamination. Refer also to Section 7.3 Decontamination procedure. Ensure that the Certificate of Decontamination as well as the return authorization number (RGA) are sent with the instrument.

The Thermo Fisher Scientific technical service department will keep you up to date with the progress of service and provide you with any further details you might need, for example, on maintenance, serviceability, troubleshooting and replacement.

9.4.1 Decontamination

Decontamination should be performed in accordance with normal laboratory procedures. Any decontamination instructions provided with the reagents used should be followed.

A decontamination procedure is only recommendable when infectious substances have been in direct contact with any part(s) of the instrument.

9.4.1.1 Certificate of Decontamination

The decontamination procedure is required prior to shipping the instrument to Thermo Fisher Scientific Oy, for example, for repair. If, for any reason, the instrument is shipped back to Thermo Fisher Scientific Oy, it must be accompanied by a dated and signed Certificate of Decontamination, which must be attached to the outside of the package containing the instrument. See Section 7.3 Decontamination procedure.

Failure to confirm decontamination will incur additional labor charges or at worst the items will be returned for proper cleaning.

Before returning any instrument(s) or item(s), ensure that they are fully decontaminated. Confirm A or B status:

Name: _____

Address: _____

Tel./Fax: _____

Name: _____ Serial no.: _____

A)

I confirm that the returned items have not been contaminated by body fluids, toxic, carcinogenic or radioactive materials or any other hazardous materials.

B)

I confirm that the returned items have been decontaminated and can be handled without exposing the personnel to health hazards.

Materials used in the unit: Chemicals + Biological • Radioactive *)

Specific information about contaminants: _____

Decontamination procedure¹: _____

Date and place: _____

Signature: _____

Name (block capitals): _____

*) The signature of a Radiation Safety Officer is also required when the unit has been used with radioactive materials.

This unit is certified by the undersigned to be free of radioactive contamination.

Date and place: _____

Signature: _____

Name (block capitals): _____

¹ Please include decontaminating solution used.

PHOTOCOPIABLE

10 Warranty Certificate

Thermo Fisher Scientific Microplate Instrumentation Business products are fully guaranteed against defective parts and materials, including defects caused by poor workmanship, for a period of one year from the date of delivery.

Thermo Fisher Scientific will repair or replace defective parts or materials during the term of warranty at no extra charge for materials and labor provided that the products were used and maintained in accordance with Thermo Fisher Scientific's instructions. The warranty is invalid if products have been misused or abused.

For the warranty to be effective, the product must have been purchased either directly from Thermo Fisher Scientific or from an authorized Thermo Fisher Scientific distributor. The guarantee is not transferable to a third party without prior written approval from Thermo Fisher Scientific.

This guarantee is subject to the following exclusions:

- Any defects caused by normal wear and tear.
- Defects caused by fire, lightning, flood, earthquake, explosion, sabotage, war, riot, or any other occurrence of the type listed above.
- Refurbished products that are subject to different warranty conditions.

THIS WARRANTY IS IN LIEU OF ALL OTHER EXPRESSED OR IMPLIED WARRANTIES, INCLUDING BUT NOT LIMITED TO ANY IMPLIED WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. The seller is not liable for any loss or damage arising out of or in connection with the use of the product or other indirect damages.

These warranty terms and conditions can be obtained from your local Thermo Fisher Scientific dealer.

10.1 Warranty limitations

Consumables are not included in the warranty.

The warranty does not extend beyond the expiration date of the reagent kit.

11 Ordering Information

Code	Instrument/System
-------------	--------------------------

5400000	KingFisher, 100 – 240 V
---------	-------------------------

11.1 List of accessories

Code	Item	Quantity
1507730	KingFisher User Manual	1
97002070	KingFisher tip comb RNase free	50 pcs/box
97002080	KingFisher plate 100 μ l RNase free	50 pcs/box
97002084	KingFisher plate 200 μ l RNase free	50 pcs/box
97002090	KingFisher plastics 8-pack, 8 plates 100 μ l + 8 tip combs/box	1 box
97002094	KingFisher plastics 200 μ l 8-pack, 8 plates + 8 tip combs/box	1 box
4540000	FP-Stepper 10 μ l – 5 ml	1
9404190	Finntip 2.5 ml	100 pcs/box

11.2 List of spare parts

Code	Item	Quantity
11272711	Plate tray	1
2305290	Serial cable F9/F25 (for RS-232C port)	1
5186980	KingFisher Software	1

11.3 List of recommended spare parts

Code	Item*	1 – 2 unit(s) / year	10 units / year
1091910	Tooth belt T2.5/330		1
1092250	Tooth belt T2.5/265		1
2004460	PCB PSP-01		1
2004470	PCB CSU-02	1	2
2004480	PCB CSU-01		1
21040811	Power supply	1	2
21040820	H-motor assembly		1
21040850	M-motor assembly		1
24070740	Free wheel assembly	1	3
24072290	Magnetic plate assembly		1

* = Contact the Thermo Fisher Scientific technical service department

12 Glossary and Abbreviations

Frame	The frame where the special microstrips are placed.
Magnetic rod	The rods which are magnetic and collect magnetic particles. The rods do not collect the particles on their own, the magnetic rods must always be protected by a tip comb.
Microstrip (or microwell strip)	The wells where all the reagents and samples are located and where the processing takes place.
Plate	Frame + microstrips (12 pieces).
Tip comb	Protects the magnetic rod. A tip comb always has to be fitted into the tip comb holder slot during processing.

12.1 Keywords for web pages

affinity separation	PCR
amplification	PCR amplification
beads	PCR product
cloned DNA	post PCR purification
cloning	primer
DNA	probes
DNA complex	processing
DNA immobilization	processor
DNA purification	purification
DNA strand separation	RNA
DNA template	mRNA
double-stranded DNA	RT-PCR
dsDNA	separation
encapsulated magnetic microspheres	separator
genome	silica-coated beads
genomic DNA	silica-coated microspheres
hybride selection	silica-coated particles
KingFisher (or Kingfisher)	streptavidin-coated beads
magnetic beads	streptavidin-coated microspheres
magnetic microspheres	streptavidin-coated particles
magnetic particles	subtraction libraries
magnetic separation	superparamagnetic beads
microspheres	superparamagnetic microspheres
nucleotide	template
oligo-dT	Thermo Fisher Scientific
particles	Thermo Scientific

12.2 Literature

Aasheim, H. – C., Deggerdal, A., Smeland, E. B. and Hornes, E. (1994): A simple subtraction method for the isolation of cell-specific genes using magnetic mono-disperse polymer particles. *Biotechniques* **16** (4), 716–721.

Coche, T., Dewez, M. and Beckers, M. – C. (1994): Generation of an unlimited supply of subtracted probe using magnetic beads and PCR. *Nucleic Acids Res.* **22**, 1322–1323.

Cudjoe, K. S. (1999): Immunomagnetic particle based techniques: Overview. In: Eds: Robinson, R. K., Batt, C. A and Patel, P. D. *Encyclopaedia of Food Microbiology*. Academic Press, London, UK. Pp. 1088–1094.

Hornes, E. and Korsnes, L. (1990): Magnetic DNA hybridisation properties of oligonucleotide probes attached to superparamagnetic beads and their use in the isolation of poly(A) mRNA from eukaryotic cells. *Genet. Anal. Tech. Appl. (GATA)* **7**, 145–150.

Jakobsen, K. S., Breivold, E. and Hornes, E. (1990): Purification of mRNA directly from crude plant tissues in 15 minutes using oligo dT microspheres. *Nucleic Acids Res.* **18**, 3669.

Jakobsen, K. S., Haugen, M., Sæboe – Larsen, S., Hollung, K., Espelund, M. and Hornes, E. (1994): Direct mRNA isolation using magnetic Oligo (dT) Beads: A protocol for all types of cell cultures, animal and plant tissues. In: Eds: Uhlén, M., Hornes, E. and Olsvik, Ø. *Advances in Biomagnetic Separation*. Eaton Publishing. Pp. 61–71.

Lambert, K. N. and Williamson, V. M. (1993): DNA library construction from small amounts of RNA using paramagnetic beads and PCR. *Nucleic Acids Res.* **21**, 775–776.

Lee, Y. – H., and Vacquier, V. D. (1992): Reusable cDNA libraries coupled to magnetic beads. *Anal. Biochem.* **206**, 206–207.

Rudi, K., Kroken, M., Dahlberg, O. J., Deggerdal, A., Jakobsen, K. S. and Larsen, F. (1997): Rapid, universal method to isolate PCR-ready DNA using magnetic beads. *BioTechniques* **22**, 506–511.

Sinclair, B. (1998): To bead or not to bead. Applications of magnetic bead technology. *Scientist* **12**, 16–19.

13 Index

A

Abbreviations	36
About the User Manual	7
Accessories.....	15, 35
Addresses	47
Advantages of processing magnetic particles.....	9
Application.....	5, 6, 7, 31, 37, 42, 46, 48
Application protocol	
KingFisher genomic DNA purification protocol for blood.....	42
KingFisher total RNA purification.....	43

B

Brief User's Guide.....	45
-------------------------	----

C

Cable	18, 24, 35
Certificate of Decontamination	25, 32, 33
Check	15, 18, 30, 32
Clean	16, 23, 24, 31, 33
magnetic rods	23
processing chamber	23
Concentration	13, 14, 44

D

Decontamination	25, 32, 33
Certificate of	25, 32, 33
Dimensions.....	26
Directives	28
Disclaimer	5
Disinfection procedure.....	22, 23, 24, 25, 32, 33
Disposal of instrument.....	25
Disposal of materials.....	24
DNA.....	9, 21, 31, 36, 37, 42

E

Environmental requirements	16
Error message	7, 29

F

FAQ	31
Feedback Form.....	15, 46
Frame	30, 36
Frequently asked questions.....	31

G

Glossary	36
----------------	----

H

How to clean the magnetic rods.....	23
How to clean the plate carrier.....	23
How to clean the processing chamber	23
How to disinfect the instrument	22, 23, 24, 32, 33
How to ensure startup.....	20
How to install the KingFisher	15, 27
How to maintain the instrument.....	23
How to pack for service	25, 32
How to release the transport lock	16
How to setup the instrument before you put it into operation.....	16
How to shut down.....	22
How to switch on.....	19, 21
How to troubleshoot the instrument.....	30
How to unpack.....	15
How to use the keypad and display	19
How to work with a magnetic rod	13

I

Incubation	9, 13, 14, 26, 43
Installation.....	7, 15, 16, 25, 27
Instrument layout	10
Intended use	8
Inverse magnetic particle processing	12, 13

K

Keypad and display.....	8, 12, 19, 45
Keys.....	19, 26
Keywords for web pages	36
Kit	8, 34, 46

L

Liquid crystal display.....	19
Literature.....	37

M

Magnetic particle processor	7, 8, 9, 12, 13, 31
Magnetic particles	8, 9, 12, 13, 14, 31, 36, 43
collection of.....	13
release of.....	13
wash of.....	13, 14
Magnetic rod	8, 11, 12, 13, 14, 23, 26, 30, 31, 36
Mains.....	11, 18, 24, 26, 27
Maintain	23, 25, 45
Maintenance	7, 23, 25, 32
Microstrip.....	8, 12, 21, 26, 30, 36

O

Operational check.....	18
Ordering information	7, 35

P

Packing.....	15, 25, 32
instructions.....	15, 25
list	15
materials.....	25
PCR.....	31, 36, 37

Plate	8, 11, 12, 20, 21, 22, 23, 24, 30, 31, 35, 36, 43, 44, 45
carrier	12, 20, 22, 23, 24, 45
Plate carrier.....	12, 20, 22, 23, 24, 45
Processing head	12
Protocol.....	8, 19, 31, 32, 37, 42, 43, 45
R	
Releasing the transport lock	16
RNA.....	36, 37, 44
RT-PCR.....	36
S	
Samples	8, 9, 12, 16, 21, 24, 26, 31, 36, 42, 43, 44, 45
Service	25, 26, 30, 31, 32
contracts.....	25
pack for.....	25, 32
request protocol	32
Shutdown	22
Specifications	7, 26, 27
general.....	26
performance.....	26
safety	27
Startup	18, 19, 21
Streptavidin-coated magnetic particles	36
Symbols	6
T	
Table of Figures.....	41
Tip comb.....	8, 11, 12, 13, 14, 20, 21, 22, 26, 30, 31, 35, 36, 45
Transport	15, 16, 17, 25, 26
damage.....	15
lock	16, 17, 25
package	15, 25
Troubleshooting	7, 29, 30, 32
guide.....	30
U	
Unpack	15
User manual.....	7, 15, 35, 46
V	
View	10, 11
front.....	10
internal.....	11
rear	11
Voltage	16, 18, 19, 26, 28
selector.....	18, 19
W	
Warnings.....	16, 17, 18, 29
Warranty	7, 15, 34
Weight.....	16, 26, 44

14 Table of Figures

<i>Fig. 3.1 KingFisher magnetic particle processor</i>	8
<i>Fig. 4.1 KingFisher front view with lids closed</i>	10
<i>Fig. 4.2 KingFisher front view with lids open</i>	10
<i>Fig. 4.3 KingFisher rear view</i>	11
<i>Fig. 4.4 KingFisher internal view</i>	11
<i>Fig. 4.5 KingFisher magnetic particle processor</i>	12
<i>Fig. 4.6 Inverse magnetic particle processing</i>	13
<i>Fig. 4.7 Concentration step during magnetic particle processing</i>	14
<i>Fig. 5.1 KingFisher transport lock tag present before release of the lock piece</i>	17
<i>Fig. 5.2 Location of the transport lock screws</i>	17
<i>Fig. 5.3 Connecting the mains supply cable</i>	18
<i>Fig. 6.1 KingFisher keypad</i>	19
<i>Fig. 6.2 Loading the plate carrier</i>	20
<i>Fig. 6.3 Positioning of the plate(s) and insertion of the tip comb(s)</i>	20
<i>Fig. 6.4 Tip comb specially designed for the KingFisher</i>	21
<i>Fig. 6.5 Plate containing microstrips specially designed for the KingFisher</i>	21
<i>Fig. 16.1 KingFisher keypad</i>	45

15 Appendix A. Application Protocols for KingFisher

A.1 Example of a KingFisher genomic DNA purification protocol from blood

The genomic DNA isolation kit based on paramagnetic particles can be used with the KingFisher magnetic particle processors to automate genomic DNA purification. Blood can be used as starting material for the DNA purification with the KingFisher. The isolated DNA can be used for several downstream applications, including PCR and restriction analysis.

The gDNA purification has been adopted into the KingFisher microplate format. The kit reagents are pipetted into the KingFisher plate. After filling the KingFisher microplate, insert the plate into the KingFisher instrument and choose the correct program by using the arrow keys. The KingFisher program is called **Genomic_DNA_1**. Start the program by pressing the **START** button. Purified genomic DNA is released into a 40 μ l volume into the last well H and the particles are returned into well F. This genomic DNA purification protocol takes ca. 30 minutes.

You can use < 40 μ l of blood as starting material.

The description of the KingFisher protocol is as follows:

Program

1. The sample is incubated with particles in well A for 3 minutes.
2. The sample is incubated with particles in well B for 3 minutes.
3. The sample is incubated with particles in well C for 3 minutes.
4. The sample is incubated with particles in well D for 3 minutes.
5. The particles are washed in well E.
6. The particles are washed in well F.
7. The particles are washed in well G.
8. The purified DNA is released into distilled water in well H.
9. The particles are removed and returned into well F.

Volumes

- A. Blood **10 μ l** + lysis buffer **75 μ l** + particles **16 μ l**
- B. Blood **10 μ l** + lysis buffer **75 μ l**
- C. Blood **10 μ l** + lysis buffer **75 μ l**
- D. Blood **10 μ l** + lysis buffer **75 μ l**
- E. Washing buffer **100 μ l**
- F. 70% EtOH **100 μ l**
- G. 70% EtOH **100 μ l**
- H. Distilled water **40 μ l**

- You can make a master mixture of the blood and lysis buffer: 40 μ l of blood + 300 μ l of lysis buffer. Then you dispense 85 μ l of the mixture into wells A – D.

A.2 Example of a KingFisher total RNA purification protocol

The total RNA purification kit based on paramagnetic particles can be used for the purification of total RNA from cultured cells or tissue homogenates. The reagents and specific consumables are designed to be used with the KingFisher magnetic particle processors as part of an integrated system.

The paramagnetic silica particles bind RNA in the presence of chaotrophic salts. After the binding step, the RNA/magnetic particle complex is washed extensively in washing buffers to remove any remaining contaminants. Finally the purified RNA is released into the elution buffer. During the elution step, the samples are heated outside the KingFisher instrument. With this protocol processing time for 24 samples is 40 minutes.

The isolated RNA can be directly used in downstream applications, such as RT-PCR and Northern blotting.

You can use 1×10^6 cells or 3 – 10 mg of tissue for the purification. Yields will vary according to the kit, cell and tissue type used in the assay.

The name of the purification protocol is **Total_RNA_1**.

Pipetting the reagents / KingFisher

Well	KingFisher microplate, 100 μl
A	Cell lysate OR tissue homogenate 100 μl + magnetic particles 10 μl
B	Washing buffer 1 100 μl
C	Washing buffer 1 100 μl
D	Washing buffer 1 100 μl
E	Washing buffer 2 100 μl
F	Washing buffer 2 100 μl
G	Washing buffer 2 100 μl
H	Elution buffer 40 μl

Protocol Total_RNA_1

1. The sample is incubated with magnetic particles in well A for 18 minutes.
2. The particles are washed in washing buffer 1 in well B.
3. The particles are washed in washing buffer 1 in well C.
4. The particles are washed in washing buffer 1 in well D.
5. The particles are washed in washing buffer 2 in well E.
6. The particles are washed in washing buffer 2 in well F.
7. The particles are washed in washing buffer 2 in well G.
8. The particles are released into the elution buffer in well H.
9. The protocol is paused for a heating step. The text marked "PAUSED" appears on the screen.
10. The plate is removed and transferred to the incubator for the heat incubation.
11. After incubation, the plate is returned to the KingFisher.
12. The protocol will continue after you press **START**.
13. The particles are removed and returned into well E.

Sample preparation from cultured cells

- Transfer cultured cells suspended in PBS into a suitable centrifuge tube.
- Collect the cells by centrifugation for 5 minutes at 6000 rpm and discard the supernatant.
- If frozen cells are used, thaw the cells on ice, centrifuge the cells for 5 minutes at 1000 rpm and discard the supernatant.
- Add lysis buffer (including 10 μ l β -ME/1000 μ l buffer) so that the final concentration is 1×10^6 cells/100 μ l.
- Mix well by vigorously pipetting up and down several times until the lysate is homogenous. Vortex for an additional 30 seconds at full speed.

Note: If a lot of foam has formed, keep the sample on ice while filling the KingFisher microplate with other reagents or centrifuge the sample for 1 minute at 1000 g (~ 3000 rpm in a microcentrifuge).

Note: If a centrifuge is used, ensure that you follow the centrifugation instructions. Do not exceed the time and speed, as this will drop the RNA yield drastically.

- Mix the homogenate gently by pipetting and add the cell mixture into the KingFisher plate according to the instructions.

Sample preparation from tissue

- Weigh the needed amount of tissue (up to 10 mg) and add 300 μ l of lysis buffer (including 10 μ l β -ME/1000 ml buffer). Depending on the rotor-stator homogenizer (for example, IKA Ultra Turrax, Heidolph DiAx, etc.) and the tool used, change the amount of tissue and lysis buffer using the same ratio as above. Tools with a diameter of 5 mm or 7 mm are suitable for small volumes (a minimum of 300 μ l) and can be used for homogenization in microfuge tubes. Refer to your homogenizer supplier for suitable handling volumes.
- Homogenize the sample on ice in the presence of lysis buffer for 5 – 90 seconds, depending on the toughness of the tissue.
- Centrifuge the homogenate for 1 minute at 1000 g (~ 3000 rpm in a microcentrifuge).

Note: Ensure that you follow the centrifugation instructions. Do not exceed the time and speed, as this will drop the RNA yield drastically.

Note: If a rotor-stator homogenizer is not available, other homogenization methods can be used instead. For example, soft tissues, such as liver, can be homogenized using, for example, a pestle and needle/syringe method. Always follow the centrifugation instructions above, despite the homogenization method used.

- Add up to 100 μ l of the supernatant into the KingFisher plate according to the instructions.

16 Appendix B. Thermo Scientific KingFisher Brief User's Guide

- Switch the KingFisher on (p. 18).
- Pipette the samples, reagents and washing buffers into the microwells of the plate(s) (p. 42).
- Load the plate(s) onto the plate carrier (p. 20).
- Insert the tip comb(s) into the tip comb holder slot(s) (p. 20).
- Select the desired protocol from the keypad and display (p. 19).
- Press the **START** button (p. 20).
- After the end of the run, remove the plate and tip combs (p. 22).
- Maintain your KingFisher instrument regularly (p. 23).



Fig. 16.1 KingFisher keypad

Please send to Thermo Fisher Scientific Oy: Fax +358-9-32910415

17 Appendix C. Thermo Scientific KingFisher Feedback Form

Cat. no.	Serial no.				
PURCHASED BY	PURCHASED FROM				
Company/Institute	Distributor				
Department	Address				
Address					
Tel.		Tel.			
Fax		Date of delivery			
Internet home page					
Date of purchase					
Your research area					
Dr. <input type="checkbox"/> Mr. <input type="checkbox"/> Mrs. <input type="checkbox"/> Ms. <input type="checkbox"/> Job title/Position					
Surname (block capitals)	First name (block capitals)				
Internet e-mail address					
	Excellent	Above expectations	As expected	Below expectations	Comments
Reagent kit/Instructions	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Instrument/User manual	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Operational reliability	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Design	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Ease of use	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Operational costs	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Customer support	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Further instrument/system developments desired:					
Further applications desired:					
Where did you first learn about the product?					
Would you like to receive information about other Thermo Scientific products?					

18 Appendix D. Addresses

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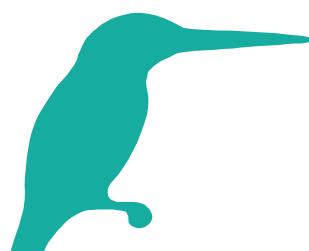
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19 Appendix E. Supplementary Application Protocols

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