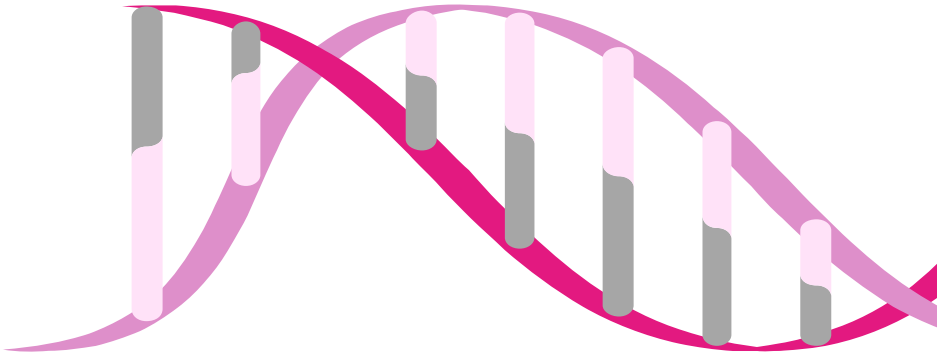
**For professional use only**



ULISSE BioMed

*We build using DNA: the molecule of life*

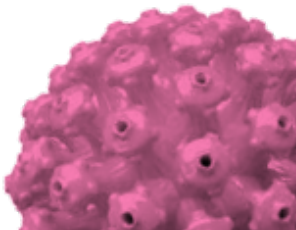
# Ulisse Faster DNA

## INSTRUCTIONS FOR USE

**Pretreatment buffer for direct PCR**

CE IVD

REF UBM0014-050



Version 5 | August 2023

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# INDEX

# 1. Product description

## Intended use

"Ulisse Faster DNA" is a pretreatment buffer (named Buffer P) that enables direct loading of vaginal swab and liquid based cervical cytology specimens in real-time polymerase chain reaction (PCR) after a brief thermal treatment step, without the need of any prior DNA purification step. The buffer has been validated through the treatment of vaginal swabs and cervical specimens collected in ThinPrep® media using an endocervical brush/spatula cervical brushes in combination with UBM0002 and UBM0013 products; however, Ulisse Faster DNA could be used in conjunction with several real-time PCR kits, upon dedicated validation. Ulisse Faster DNA is intended for use by clinical laboratory personnel trained in real-time PCR techniques and in vitro diagnostic procedures.

## Principles and procedure overview

Ulisse Faster DNA is composed by a pretreatment buffer, that has to be added 1:5 to biological samples. The sample premixed with the buffer is incubated at two different temperatures using a thermomixer. During this process, nucleic acid DNA are solubilized. After pretreatment the sample is ready to be loaded in compatible PCR reactions. For a good performance, the specimen must be collected and stored strictly following the manufacturer's instructions for use.

Other Real Time PCR instruments and IVD assays may be compatible; however, an end user validation will be required before use.

All instruments used must be installed, calibrated, checked, and maintained according to the manufacturer's instructions for optimal results.

## Storage and handling

Ulisse Faster DNA should be stored at a temperature between -25 °C and -15 °C, in an upright position and away from light. Ulisse Faster DNA is stable under recommended storage conditions until the expiry date stated on the label. Repeated thawing and freezing should be avoided, as this may reduce the sensitivity. Ulisse Faster DNA can be frozen and thawed for no more than 6 times; further freezing/thawing cycles may cause a loss of product performance. If the reagents are to be used only intermittently, they should be frozen in aliquots in RNase/DNase free tubes.

## Materials provided

The material contained in one kit of Ulisse Faster DNA (Ulisse Biomed, S.p.A.; code #UBM0014-050) are sufficient for the pretreatment of 50 samples.

| Ulisse Faster DNA ( REF UBM0014-050) | | | |
|---|---|---|---|
| Contents | Volume | Description | Color |
| Buffer P | 1 X 1.20 mL | Pretreatment buffer. | Blue |

## Materials required but not provided

### A. Materials required for every compatible system:

The following materials are required to use Ulisse Faster DNA on every compatible system:

- molecular-biology grade water, RNase and DNase free.
- 1x Phosphate Buffered Saline solution (PBS).
- Precision calibrated pipettes capable of dispensing 2-20 µl (0.1-0.2 µl increment), 20-200 µl (0.1-0.2 µl increment), and 100-1,000 µl (1-2 µl increment).
- Anti-aerosol, single use, low-retention sterile filter tips for precision pipettes of 2-20 µl, 20-200 µl, and 100-1,250 µl, nuclease free.
- Desktop centrifuge.
- Vortex mixer.
- Class II laminar airflow biological hood.
- Ice.
- Disposable nitrile powder-free gloves, or similar material, and adequate personal protective equipment.
- Disinfectant surface cleaner.

### B. Materials required for pretreatment performed in a thermomixer

For pretreatment in a thermomixer, the following materials are required:

- thermomixer.
- 1.5 mL polypropylene capped tubes, sterile, RNase and DNase free.

### C. Materials required for pretreatment performed in a PCR thermocycler

For pretreatment in a PCR thermocycler, the following materials are required:

- 96 multi-well PCR plates (well volume not inferior to 0.2 ml) or 8-tube strip tubes or PCR tubes.
- Adhesive seal or 8-tubes strip caps compatible with the PCR plate/tubes.
- PCR sealer.
- PCR thermocycler.

# 2. Warnings and precautions

This product is exclusively designed for *in vitro* use.

## General warnings and precautions

- Handle and dispose of all biological samples as if they were able to transmit infective agents. Avoid direct contact with the biological samples. Avoid splashing or spraying. The materials that come into contact with the biological samples must be treated for at least 30 minutes with 3% sodium hypochlorite or autoclaved for one hour at 121 °C before disposal.
- Handle and dispose of all reagents and all materials used to carry out the assay as if they were able to transmit infective agents. Avoid direct contact with the reagents. Avoid splashing or spraying. Waste must be handled and disposed of in compliance with adequate safety standards.
- Wear suitable protective clothes and gloves; protect eyes and face.
- Never pipette solutions by mouth.
- Do not eat, drink, smoke or apply cosmetic products in the work areas.
- Carefully wash hands after handling samples and reagents.
- Dispose of leftover reagents and waste in compliance with the regulations in force.
- Carefully read all the instructions provided with the product before running the assay.
- While running the assay, follow the instructions provided with the product.
- Do not use the product after the indicated expiry date.
- Do not use the product if, upon receipt, the package is damaged, or the seal is broken.
- Only use the reagents provided with the product and those recommended by the manufacturer.
- Do not pool reagents from different lots or from different tubes of the same lot.
- Do not use reagents from other manufacturers.
- Be aware that you may be required to report serious incidents that have occurred in relation to the device to the manufacturer and the regulatory authority in which the user and/or the patient is established.
- If liquid containing the Buffer P is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite. If the buffer tubes are damaged or leaking, wear gloves and protective goggles when discarding the bottles in order to avoid personal injury or injury to others.
- Ulisse Biomed has not tested the liquid waste generated by the Ulisse Faster DNA procedures for residual infectious materials. Contamination of the liquid waste with residual infectious materials is unlikely, but cannot be excluded completely. Therefore, liquid waste must be considered infectious and be handled and discarded according to local safety regulations.
- For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at www.ulissebiomed.com/docs, where you can find, view and print the SDS for each Ulisse Biomed kit.

## Warnings and precautions for molecular biology

- Molecular biology procedures require qualified and trained staff to avoid the risk of erroneous results, especially due to the degradation of nucleic acids contained in the samples or sample contamination.
- Lab coats, gloves and tools dedicated to work session setup are needed.
- The samples must be suitable and, if possible, dedicated for this type of analysis. Samples must be handled under a laminar airflow hood. Pipettes used to handle samples must be exclusively used for this specific purpose.
- DNA and RNA samples are susceptible to degradation by DNAses and RNAses, respectively. Ensure all specimens are handled with appropriate laboratory DNA/RNA handling procedures including using DNAse- and RNAse-free tubes and tips.
- Minimize the possibility of cross-contamination between the samples themselves. All dispensing steps must be performed using pipettes with disposable anti-aerosol filter tips, to be replaced at each liquid transfer.
- Plate sealing must follow manufacturer's indication aiming to avoid well-to-well contamination.

# 3. Protocol

## Specimen collection, storage and transport

### A. Specimen Collection

*Liquid based cervical cytology specimen*

Cervical specimen collected in ThinPrep® media using an endocervical brush/spatula has been validated for use with Ulisse Faster DNA. Follow the manufacturer's instructions for collecting cervical specimen.

*Vaginal swab specimen*

For the self-collection of vaginal swab specimen, please use following materials according to manufacturer's instructions:

- FLOQSwab® regular plus, rounded tip, peelable barcode, no breaking point (Copan Italia, S.p.A.; code #5E046S) for self-collection of vaginal specimens.

### B. Specimen Storage

The sensitivity of the assay may decrease if specimen is repeatedly frozen and thawed or stored for a long period of time. Nucleic acids should be extracted from the specimen as quickly as possible.

*Liquid based cervical cytology specimen*

Cervical cell specimen collected in ThinPrep® medium may be stored at 2 ~ 8 °C for up to 6 weeks.

*Vaginal swab specimens*

If the cervical and vaginal swab specimens are not processed directly after their receipt in the laboratory, they have to be stored at -15° ~ -25 °C and have to be processed within one month.

### C. Specimen Transport

To ensure a high quality of sample, specimens should be transported as soon as possible at indicated temperature.

*Liquid based cervical cytology specimen*

Cervical cell specimen collected in ThinPrep® medium can be transported at 2 ~ 25 °C.

*Vaginal swab specimens*

Vaginal swab specimens shall be preferably transported cooled, but they can be transported at room temperature (~ + 25 °C) for a period no longer than 7 days. Vaginal swab specimens should be shipped to a laboratory as soon as possible after collection, following the laboratory instructions for transports. The samples should be transported following also the local and national instructions for the transport of pathogen material.

## Procedure

The procedure to use Ulisse Faster DNA consists of two steps:

A. sample preparation.

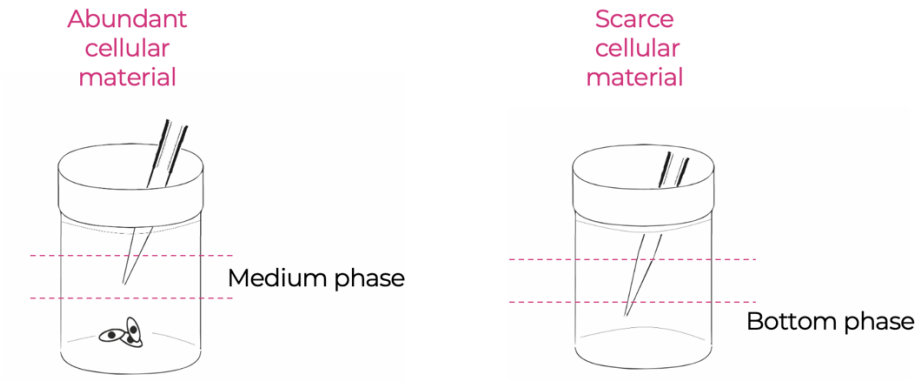
B. Pretreatment.

### A. Sample preparation

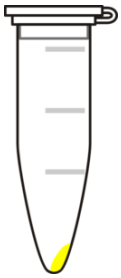
#### *a. Preparation of liquid based cervical cytology specimens*

Before pretreatment with Ulisse Faster DNA, liquid based cervical cytology specimens stored in Thin Prep® have to be prepared as indicated hereby:

- vortex the Thin Prep® vial for at least 30 seconds to homogenize the sample.
- Transfer 1.5 mL of liquid based cervical cytology specimen from the original Thin Prep® vial into a 1.5 mL tube. In case of cellular material-rich sample, take the aliquot from the middle phase avoiding the aspiration of cell lumps. In case of cellular material-poor sample, take the aliquot from the bottom phase instead.




- Centrifuge the tube at >9,000 g for 9 minutes.
- Remove the supernatant manually with the pipette, taking care not to aspirate the cell pellet. Excessive leftover of Thin Prep® solution could cause inhibition of the following PCR reaction. The correct pellet size to achieve is depicted in the image below. In case of smaller or bigger pellet, refer to the "Troubleshooting" paragraph.



- Add 1 mL of 1x Phosphate Buffered Saline solution (PBS) to the cell pellet and place the tube on the vortex for at least 30 seconds.
- Centrifuge the tube at >9,000 g for 9 minutes.
- Remove the supernatant manually with the pipette, taking care not to aspirate the cell pellet.
- Resuspend in 80 µL of molecular-biology grade water.

The prepared samples can be now pretreated with Ulisse Faster DNA.

### b. *Preparation of vaginal swab specimens*

Before pretreatment with Ulisse Faster DNA, vaginal swab specimens have to be resuspended as indicated hereby:

- use a pipette with a disposable tip to transfer 2 mL of molecular-biology grade water into the 5 mL tube.
- Immerse the swab in the water with a series of rapid vertical movements; subsequently and without being immersed, the plug must be rotated by pressing it against the walls of the tube in order to facilitate the release of as much material as possible.
- Make the suspension homogeneous by vortexing it for 10-20 seconds so that no precipitate is visible.
- The prepared samples can be now pretreated with Ulisse Faster DNA.

### c. *Compatible instruments*

The thermomixer has to be able to reach the temperature of 56°C and >98°C shaking with a speed of >300 rpm. The thermocycler has to be able to reach the temperature of 56°C and >98°C.

The following instruments have been validated for use with Ulisse Faster DNA:

- Thermomixer® C (Eppendorf, AG; code #5382).
- QuantStudio™ 5 Real-Time PCR System (Applied Biosystems, Inc.).

## B. Pretreatment

### a. *Pretreatment using a thermomixer*

Thaw the reagents at room temperature (~ + 25 °C) for 30 minutes. Mix gently, spin down the content for 5 seconds. Keep all the reagents on ice during the preparation.

Prepare one 1.5 mL polypropylene capped tube for each sample to be treated and identify the tube with an indelible marker.

Use a pipette with disposable anti-aerosol tip to transfer 80 µL of the prepared samples into the test tube.

Use a pipette with disposable aerosol tip to transfer 20 µL of Buffer P into the tube in order to have a final volume of 100 µL. The resulting 100 µL must be mixed briefly with a vortex, spin down with a centrifuge and left to react on a thermomixer for 10 minutes at 56 ° C under stirring at 400 rpm and then for 10 minutes at 98°C -100° C. After chilling the tube, spin it at >9,000 g for 3 minutes and use supernatant to load the Real Time PCR. Residual volumes of untreated biological samples can be stored between -15 ° C and -25 ° C for up to 1 month.

### b. *Pretreatment using a PCR thermocycler*

Thaw the reagents at room temperature (~ + 25 °C) for 30 minutes. Mix gently, spin down the content for 5 seconds. Keep all the reagents on ice during the preparation.

Use a pipette with disposable anti-aerosol tip to transfer 80 µL of each prepared samples into one well of the 96 multi-well PCR plate or in a 8-tube PCR strip or in single PCR tube.

Use a pipette with disposable aerosol tip to transfer 20 µL of Buffer P into each well in order to have a final volume of 100 µL.

Seal the plate with adhesive film or tube caps according manufacturer's instructions for use and load the plate in the PCR instrument.

Run the following protocol: 10 minutes at 56 ° C and then for 10 minutes at 98°C -100° C. After chilling the tube, spin it at >9,000 g for 3 minutes and use supernatant to load the Real Time PCR.

Residual volumes of untreated biological samples can be stored between -15 ° C and -25 ° C for up to 1 month.

| **Sample : Buffer Ratio** | |
|---|---|
| Component | Volume |
| Sample | 80.00 µL |
| Buffer P | 20.00 µL |
| Total Volume | 100.00 µL |

## Quality control

Quality Control In accordance with Ulisse BioMed ISO-certified Quality Management System, each lot of Ulisse Faster DNA is tested against predetermined specifications to ensure consistent product quality.

## Troubleshooting

| Issue / Error | Possible cause | Possible solution |
|---|---|---|
| Invalid results in the downstream PCR application | Pipetting error. | Take care when dispensing reagents. |
| | Pretreatment setup error.<br>-<br>Inadequate pretreatment | Verify to have executed correctly the instructions described in the paragraph "Procedure".<br>Repeat pretreatment. |
| | Inadequate storage of reagents. | Use a new aliquot of reagents or a new kit. |
| | DNase presence. | Use DNAse-free consumables. |
| | Inadequate sample. | Verify sample compatibility and adequacy. |
| | Scarce amount of sample. | In case of liquid based cytology specimen, pellet a bigger amount of sample. |
| | Excessive amount of sample.<br>-<br>PCR inhibitors presence. | Try to dilute the pre-treated sample 1:5. |
| | Inadequate sample collection, storage or transport. | Repeat pretreatment or sample collection. |
| | Chemical contamination. | Try to dilute the pre-treated sample 1:5.<br>With liquid based cytology specimen repeat the preparation doubling the PBS washing step and take care of removing most of the solution. |
| Contamination in the downstream PCR application | Local contamination. | Clean sample preparation area.<br>Ensure that adequate Personal Protection Equipment are used to reduce contamination risk. |
| | Reagent contamination. | Use a new aliquot of Buffer P. |
| | Pipetting error. | Always change tip between samples.<br>Take care when dispensing reagents and samples. |
| | Pretreatment setup error. | Verify to have executed correctly the instructions described in the paragraph "Procedure". |
| | Sealing error. | Take care when sealing the plate or the tube and follow the manufacturer's instructions. |

## 4. Limits

The system performance has been established using vaginal swabs and liquid based cytology specimens for isolation of genomic and viral DNA.

It is the user's responsibility to validate system performance for any procedures used in their laboratory which are not covered by the Ulisse Biomed performance studies.

To minimize the risk of a negative impact on the diagnostic results, adequate controls for downstream applications should be used.

Any diagnostic results that are generated must be interpreted in conjunction with other clinical or laboratory findings.

## 5. Explanations of symbols

Key to symbols used in the manual and labels.

| Symbol | Explanation |
|---|---|
|  | *In vitro* diagnostic medical device |
|  | Batch code |
|  | Catalogue number |
|  | Use by date |
|  | Temperature limit |
|  | Consult instructions for use |
|  | Manufacturer |
|  | Contains sufficient for <n> tests |
|  | Do not use if package is damaged and consult instructions for use |

## 6. Contacts

Contact your local Ulisse Biomed representative for assistance.

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For more contact information visit www.ulissebiomed.com