LONDON BIO FOUNDRY

Automated RNA extraction using a Felix liquid handling robot

Current version:	16
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Kits:	innuPREP Virus DNA/RNA Kit – FX (Analytik Jena) Maxwell HT Viral TNA (Promega)
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Contents

1 Preparing tips

Prepare 2 × 1000 µl tip trays:

- Prepare the 97 mm metal support (OL3317-11-105) by placing the plastic tip protector into the base (this prevents cross-contamination).
- Open the plastic tip box.
- Place the tip converter (OL3396-352-25) over the top of the tips.
- Invert the box onto a flat surface.
- Remove the white plastic insert from the tips.
- Slide the black magazine (OL3810-13-024) over the tips, with the metal rods pointing downwards.
- Invert the tips again, and remove the tip converter.
- Place the tips onto the 97 mm metal support.
- Place the supported tips into the Felix at positions 2 or 5 (centre of the lower deck).

Proceed to prepare input plates according to which kit you are using, there are minor differences so please double-check you are following the correct section.

2 innuPREP Virus DNA/RNA Kit (Analytik Jena)

Manufacturer:	AnalytikJena
Cat No:	845-KS-4800

Please note that the protocols for a 400 µl sample volume and a 200 µl sample volume are different.

Note the different volume of Carrier Mix added to Lysis Solution V.

The 200 µl protocol also uses one less plate (Plate 3). For simplicity, the plate numbering system is preserved between all protocols to aid in placement on the Felix deck.

2.1 Kit preparation

Prepare the innuPREP Virus DNA/RNA Kit contents as necessary:

- Add 1.5 ml ddH_2O to the vial of lyophilised proteinase K, mix thoroughly, store at −20 °C.
- Add 1.25 ml RNase-free water to the vial of lyophilised Carrier Mix, mix thoroughly, store at −20 °C; do not freeze/thaw more than 3 times.
- Add 72 ml absolute ethanol to Washing Solution B2, mix thoroughly.
- Vigorously shake MAG suspension, or vortex for 30 s before use.

2.2 Plate preparation – 400 µl sample volume

Make sure that you are using 400 µl sample volume, otherwise, skip to **Section 2.3: Plate preparation – 200 µl sample volume** (pg. 4)

- Add 1.3 ml Carrier Mix to 52 ml Lysis Solution V, store at 4 °C (maximum 7 days).

Prepare 96-well deep-well plates (provided in the kit):

Plate 1: Lysis/Sample

- 400 µl Lysis Solution/Carrier Mix
- 400 µl liquid sample
- 50 µl proteinase K

Plate 2: Process

- 450 µl Binding Solution V
- 50 µl MAG Suspension

Plate 3: Binding

- 450 µl Binding Solution V

Plate 4: Washing Solution A

- 1100 µl Washing Solution A

Plate 5: Washing Solution B2

- 1100 µl Washing Solution B2

Plate 6: Elution

- Leave empty

Plate 7: Water

- 600 µl RNase-free water

Prepare **Plate 8: Final elution** which can be an empty 96-well PCR plate (any 96-well labware should work, but please confirm before trying new plates).

2.3 Plate preparation – 200 µl sample volume

Make sure that you are using 200 µl sample volume, otherwise, skip to **Section 2.2: Plate preparation – 400 µl sample volume** (pg. 3)

- Add 1.75 ml Carrier Mix to 35 ml Lysis Solution V, store at 4 °C (maximum 7 days).

Prepare 96-well deep-well plates (provided in the kit):

Plate 1: Lysis/Sample

- 200 µl Lysis Solution/Carrier Mix
- 200 µl liquid sample
- 50 µl proteinase K

Plate 2: Process

- 450 µl Binding Solution V
- 50 µl MAG Suspension

Plate 4*: Washing Solution A

(*Plate 3 is omitted)

- 1100 µl Washing Solution A

Plate 5: Washing Solution B2

- 1100 µl Washing Solution B2

Plate 6: Elution

- Leave empty

Plate 7: Water

- 600 µl RNase-free water

Prepare **Plate 8: Final elution** which can be an empty 96-well PCR plate (any 96-well labware should work, but please confirm before trying new plates).

2.4 Deck layout

Place plates onto the Felix according to the scheme below.

Bottom Deck

1 Plate 1: Lysis/Sample on Bioshake	2 1000 µl tips on OL3317-11-105 (97 mm)	3 Gripper on OL3317-11-120 (37 mm)
4 Empty	5 1000 µl tips on OL3317-11-105 (97 mm)	6 Plate 6: Elution (empty) on Plate 7: Water

Top Deck

7 Plate 3: Binding solution OR empty	8 Plate 4: Washing solution A	9 Plate 5: Washing solution B2
10 Magnet adapter	11 Plate 2: Process	12 Plate 8: Final elution

Ensure that the plates are aligned with the A1 well in the top left corner and are well constrained by the metal posts at each Felix deck position.

Ensure that the Gripper is oriented with the category number label facing towards you.

3 Maxwell HT Viral TNA Kit (Promega)

Manufacturer:	Promega
Cat No:	AX2340

3.1 Kit preparation

Prepare the Maxwell HT Viral TNA Kit contents as necessary:

- Add 77 ml isopropanol to 4/40 Wash.
- Add 35 ml absolute ethanol to Alcohol Wash Buffer.
- Add 35 ml isopropanol to Alcohol Wash Buffer.
- Prepare 50 ml fresh 80 % ethanol with nuclease-free water.
- Vigorously shake MagneSil Red magnetic beads before use.

3.2 Plate preparation

Prepare 96-well deep-well plates:

Plate 1: Lysis/Sample

- 300 µl Lysis Buffer
- 200 µl liquid sample
- 30 µl proteinase K

Plate 2: Process

- 600 µl isopropanol
- 20 µl MagneSil Red

Plate 3: 4/40 Wash

- 300 µl 4/40 Wash

Plate 4: Alcohol Wash

- 300 µl Alcohol Wash

Plate 5: 80 % ethanol

- 460 µl 80 % ethanol

Plate 6: Elution

- Leave empty

Plate 7: Water

- 200 µl RNase-free water

Prepare **Plate 8: Final elution** which can be an empty 96-well PCR plate (any 96-well labware should work, but please confirm before trying new plates).

3.3 Deck layout

Place plates onto the Felix according to the scheme below.

Bottom Deck

1 Plate 1: Lysis/Sample on Bioshake	2 1000 µl tips on OL3317-11-105 (97 mm)	3 Gripper on OL3317-11-120 (37 mm)
4 Empty	5 1000 µl tips on OL3317-11-105 (97 mm)	6 Plate 6: Elution (empty) on Plate 7: Water

Top Deck

7 Plate 3: 4/40 Wash	8 Plate 4: Alcohol Wash	9 Plate 5: 80 % ethanol
10 Magnet adapter	11 Plate 2: Process	12 Plate 8: Final elution

Ensure that the plates are aligned with the A1 well in the top left corner and are well constrained by the metal posts at each Felix deck position.

Ensure that the Gripper is oriented with the category number label facing towards you.

4 Running the protocol

- Open `CyBio Composer eXtract` software from the shortcut on Desktop).
- When prompted, select the profile `with Bioshake`.
- From the `File` menu, open the `v16.bms`.
- This file can be accessed from the shortcut on the desktop to "user files" in the `Extraction_Protocols_Felix` folder.
- Turn on the Felix and ensure that the door is closed.
- Ensure the Bioshake displays a green light, indicating a good connection.
- Press the green start button.
- The protocol may request calibration, press `OK`.
- You will be asked to select which kit you are using, select appropriately.
- If using innuPREP Virus DNA/RNA Kit – FX, you will be prompted to select the sample volume you are using.
- A short confirmation will be displayed to ensure you have selected the correct kit.
- After initialisation, the protocol will ask for confirmation that you have placed the required plates in the correct locations.
- Verify that the plates are correctly positioned, and press `OK` to start the protocol.
- Wait for up to 72 min ($\sim$60 min for 200 µl sample volume).
- Remove the final elution plate from position 12 (bottom right of the top deck) for downstream processing.
- Switch off the Felix.
- Discard used labware and tips.

It is a good idea to power cycle the Felix and the Bioshake between each run (at a minimum every day) to force recalibration of the Felix.

Do not change the USB port on the laptop for the Felix connection, this can cause connectivity issues.

Frequent cleaning using RNaseZap™ or similar products is recommended.

5 Troubleshooting

In the unlikely event of system failure, the robot can be recovered from a variety of states, a number of scripts are provided to recover from such states. These scripts can be found in the `troubleshooting` directory.

Turning the Felix off then on, and closing and re-opening Composer can often fix problems not listed below. Please do not hesitate to contact me at `m.priestman13@imperial.ac.uk` if there are any other issues at all.

Bioshake ELM locked

The Edge Locking Mechanism (ELM) is the four pins on the Bioshake that lock the plate in place whilst it is shaking. In the event that the pins are locked at start-up (preventing a fresh plate from being placed), you can run the script `unlock_bioshake.bms` which should move the pins to their unlocked position.

Head blocks deck movement after shutdown

The head can be moved to the highest position by running `move_head.bms`.

Alternatively, to move the head manually, open the Felix door and hold the up button until the green light starts to flash. Release the button and press it again rapidly to start moving the head upwards. Perform the same operation with the down button to move the head downwards.

Tips still loaded

Empty the Felix, place an empty 97 mm support (OL3317-11-105) onto deck position **5**, run `unload_tips.bms`.

Gripper loaded without plate

Empty the Felix, place an empty 37 mm support (OL3317-11-120) onto deck position **3**, run `unload_gripper.bms`.

Gripper loaded with plate

Empty the Felix, place an empty 37 mm support (OL3317-11-120) onto deck position **3**, run `unload_plate.bms`.