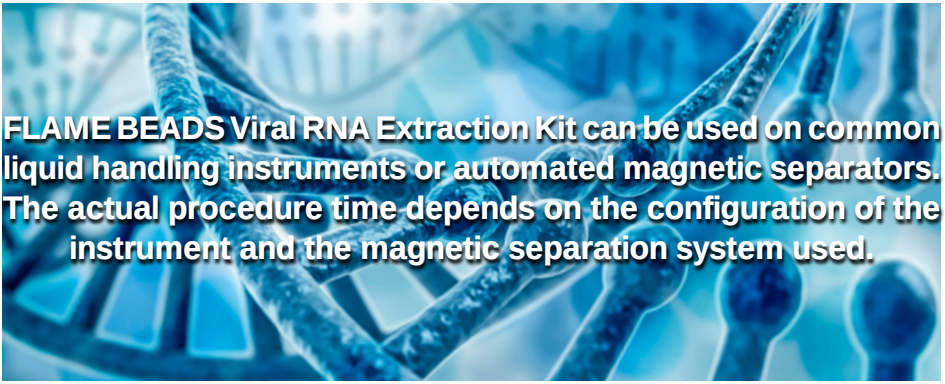
FILTER TECHNOLOGY

FLAME BEADS RNA EXTRACTION KIT

APPLICATION NOTE

PROTOCOL FOR THE ISOLATION OF VIRAL RNA ON KINGFISHER FLEX

Application note



Equipments needed for automated RNA isolation

The needed equipments may vary depending on the instrument used and are:

- Specific equipment for KingFisher instruments: Deep Well KF96 plates (Thermo scientific, 96 tests : ref : 95040450); Elution KF96 plates (Thermo scientific, 96 tests : ref : 97002540); KF 96 TIP COMBS FOR DW (Thermo scientific, 96 tests : ref :97002534)
- Ultra-Low Temperature Freezer for storage of isolated samples at -80 °C
- Biological Safety Cabinet suitable for work with potentially infectious samples.

Reagents to be supplied by the user

- Isopropanol for Molecular Biology.
- Ethanol (96-100%) for Molecular Biology
- DNase/RNase-free water

Ensure that the proper program (**MVP_Flex_200ul**) has been downloaded from the product page and loaded onto the instrument.

Before starting

Preparation of FLAME BEADS Washing Buffer 1:

FLAME BEADS Washing Buffer 1 is supplied as a concentrate. Before using it for the first time, transfer all the content of FLAME BEADS Washing Buffer 1 (concentrated) in a clean bottle (not provided) and add Ethanol (96-100%, not provided) as indicated in the following table:

Kit size	FLAME BEADS Washing Buffer 1 (concentrate)	Ethanol (96-100%) to add	FLAME BEADS Washing Buffer 1 (ready-to-use)
1x96	15 ml	45 mL	60 mL
8x96	100 ml	300 mL	400 mL
64x96	1000 ml	3000 ml	4000 ml

After Ethanol has been added, mark the bottle to indicate that this step has been completed.

Preparation of FLAME BEADS Washing Buffer 2:

FLAME BEADS Washing Buffer 2 is supplied as a concentrate. Before using it for the first time, transfer all the content of FLAME BEADS Washing Buffer 2 (concentrated) in a clean bottle (not provided) and add Ethanol (96-100%, not provided) as indicated in the following table:

Kit size	FLAME BEADS Washing Buffer 1 (concentrate)	Ethanol (96-100%) to add	FLAME BEADS Washing Buffer 1 (ready-to-use)
1x96	15 ml	60 mL	75 mL
8x96	80 ml	320 mL	400 mL
64x96	1000 ml	4000 ml	5000 ml

After Ethanol has been added, mark the bottle to indicate that this step has been completed.

Preparation of FLAME BEADS Viral lysis Buffer:

FLAME BEADS Viral Lysis Buffer may form salt precipitates upon storage. If any precipitate formed, incubate the buffer bottle at 40 °C until all of the precipitates is re-dissolved.

Preparation of FLAME BEADS magnetic beads:

Before distributing the beads, make sure that the beads are completely re-suspended. Shake the storage bottle well or place it on a vortexer shortly.

Magnetic separation time depends on the magnetic strength of the magnetic separator, distance of the separation plate from the magnetic pins, and the volume to be processed. Optimization may be required for each system.

Prepare the sample

Use a 150 µL aliquote of sample or body fluid to proceed with Step 1.

Nasopharyngeal swab /oropharyngeal swab:

For dry swab we recommend to place the dry swab in 400-500 µL of sterile PBS with gentle shaking for 30 minutes (PBS should cover completely the swab head). Use a 150 µL aliquot to proceed with Step 1.

For swab in Universal Transport Media we recommend to incubate the swab in Universal Transport Media for 30 minutes with gentle shaking to release sample material. Use a 150 µL aliquote to proceed with Step 1.

Set up the plates

Use standard 96 deep well plates compatible with KingFisher™ Flex

Set up the Wash, Elution, and Tip Comb Plates outside the instrument according to the following table:

Plate	Plate position	Component	Reagent Volume per well
Wash 1 Plate	2	FLAME BEADS Washing Buffer 1 *	500 µL
Wash 2 Plate	3	FLAME BEADS Washing Buffer 2 *	500 µL
Wash 3 Plate	4	EtOH	500 µL
Elution Plate	5	DNase/RNase free water	100 µL
Tip Comb	6	Place a 96 Deep-well Tip Comb in a Standard Plate	

*prepared as reported in the section “Before Starting”

Prepare the Sample Plate by adding, in the order, the following reagents to each well of a standard 96 deep well plate:

- 300 µL FLAME BEADS Viral Lysis buffer
- 500 µL Isopropanol
- 150 µL of sample
- 20 µL of FLAME BEADS Magnetic Beads. Be sure to mix well the bottle of FLAME BEADS Magnetic Beads before every pipetting.

Start the run

Select the program **MVP_Flex_200ul** on the instrument.
Start the run. Load the prepared plates into the indicated position when prompted by the instrument.

Collect DNA/RNA

After the run is complete (~25 minutes), remove the elution plate from the instrument.
For pre-filled plates, lifesciences.it@gvs.com

For further information

visit www.gvs.com/mag

contact lifesciences.it@gvs.com

For orders: areacsm@gvs.it

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THE ONLY WAY TO SAY FILTRATION