CANAM SCIENTIFIC

REF M10-MPOP-01

STANDARD M10 MPX/OPX

STANDARD™ M10 MPX/OPX

Instructions for Use

For use with STANDARD™ M10 system

SD BIOSENSOR

1. Intended Use

STANDARD M10 MPX/OPX test is a multiplex real-time PCR test intended for use with STANDARD M10 system for the qualitative detection of viral DNA from Monkeypox virus (MPX) in skin lesion or serum/plasma or whole blood or nasopharyngeal or oropharyngeal swab specimens collected from individuals suspected of MPX. STANDARD M10 MPX/OPX detects Monkeypox virus (MPX) and distinguishes it from other Orthopoxvirus (OPX) in a pooled result. Results are for the identification of MPX and OPX DNA. Positive results are indicative of the presence of MPX and OPX DNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Negative results do not preclude MPX infection and should not be used as the sole basis for treatment or other patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information. STANDARD M10 MPX/OPX test is intended to be performed by trained users in both laboratory and near patient testing settings.

2. Summary and Explanation

Monkeypox virus is a double-stranded DNA virus and a member of the Orthopoxvirus, a genus of the family *Poxyviridae* that contains over viral species that target mammals. Other Orthopoxvirus species pathogenic to humans include cowpox virus and variola virus, which has caused smallpox outbreak in the 20th century. MPX infection can be occurred by physical contact with an infected animal or human. Major hosts are rodents and primates, but recently, human-to-human infections are increasing. Infection occurs by contact with a symptomatic patient. Infection results in fever, headaches and muscle aches and the formation of lesions.
STANDARD M10 MPX/OPX test is a molecular *in vitro* diagnostic test that aids in the detection and diagnosis of MPX based on widely used nucleic acid amplification technology. STANDARD M10 MPX/OPX test contains primers and probes and internal control (IC) used in Real-time PCR for the *in vitro* qualitative detection of MPX and OPX DNA in skin lesion or serum/plasma or whole blood or nasopharyngeal or oropharyngeal swab specimens.

[Cartridge Description]
STANDARD M10 MPX/OPX cartridge is a disposable plastic device that allows performance of fully automated molecular assays by containing all reagents required for the test. Within the cartridge, multiple steps are automatically performed in sequence using pneumatic pressure to transfer samples and fluids via the chamber to their intended destinations.

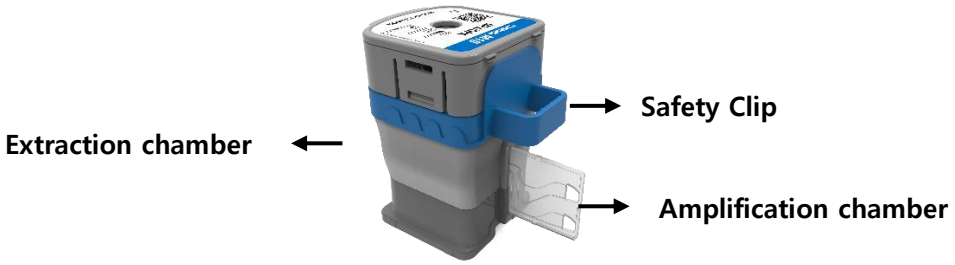

Figure 1. Layout of STANDARD M10 MPX/OPX cartridge

3. Principle of the Procedure

STANDARD M10 MPX/OPX test is an automated *in vitro* diagnostic test for qualitative detection of nucleic acid from MPX and OPX. STANDARD M10 MPX/OPX test is performed on STANDARD M10 system. STANDARD M10 system automate and integrate sample preparation, nucleic acid extraction and amplification, and detection of the target sequences in various specimens using molecular diagnostic assays. The system consists of STANDARD™ M10 Module and STANDARD™ M10 Console with preloaded software for running tests and viewing the results. The system requires the use of single-use disposable cartridges that hold the RT-PCR reagents and host the RT-PCR process. Because the cartridges are self-contained, cross-contamination between samples is minimized. For a full description of the systems, see STANDARD M10 system User Manual. STANDARD M10 MPX/OPX test includes reagents for the detection of MPX and OPX DNA in skin lesion or serum/plasma or

whole blood or nasopharyngeal or oropharyngeal swab. The cartridge is present to control for adequate processing of the sample and RT-PCR reaction.
The table below indicates which target is designed to be detected by which channel.

Table 1. Fluorescent channel of each target gene

Target	Channel
MPX	FAM
OPX	HEX
Internal control (IC)	CY5

4. Materials Provided

STANDARD M10 MPX/OPX contains sufficient reagents to process 10 specimens or quality control samples.

Table 2. Contents of STANDARD M10 MPX/OPX kit

No.	Contents	Quantity	Usage in each reaction
1	Cartridge	10	1ea
2	Quick Reference Instructions	1	-

5. Storage and Handling

Store STANDARD M10 MPX/OPX kit at 2~28℃ (36~82℉). If the cartridge has been refrigerated, perform the test after stabilizing it for 30 minutes at room temperature (20-28°C, 68~82℉). Do not remove the Safety Clip of the cartridge and do not press the cartridge until actual use. Do not use a cartridge that has leaked or is wet. Under these conditions, cartridges can be stored until the expiration date printed on the packaging.

6. Materials Required but Not Provided

- STANDARD M10 system with User Manual
  At least one STANDARD M10 Console and one STANDARD M10 Module

- Sample collection tools
  - Universal Transport Medium (COPAN)
  - BD Vacutainer® SST™ Tubes (BD)
  - VACUETTE® Serum Clot Activator Tubes (Greiner Bio-one)
  - Noble Bio CTM (Noble Biosciences : UTNFS-3B-2)
  - COPAN eNAT (Copan : 606CS01P)
  - COPAN Universal Transport Medium (recommended 3ml of UTM-RT medium)

- Sample transfer pipettes
  - STANDARD™ Fixed volume dropper (300*μl*)
  - Micropipette with filter tips

- PPE (Personal Protective Equipment)

- Biohazard container

7. Warnings and Precautions

1) This kit is only for *in vitro* diagnosis.
2) Please read the Instructions for Use carefully before testing.
3) Improper specimen collection, transfer, storage, and processing may cause erroneous test results.
4) Do not remove the Safety Clip of the cartridge before use.

5) Do not press the cartridge until actual use.
6) Do not use a cartridge that has leaked or is wet.
7) Do not use the kit after its expiration date.
8) Do not shake, tilt, or invert the cartridge especially after pressing the cartridge to punch the seal. It may yield non-determinate results.
9) Do not use a cartridge with a damaged barcode label.
10) Do not reuse processed cartridges.
11) All patient samples should be handled as if these samples are infectious.
12) All materials should be considered potentially infectious and should be handled with precautions.
13) As this test involves extraction of viral DNA and PCR amplification, care should be taken to avoid contamination. Regular monitoring of laboratory contamination is recommended.
14) Clinical laboratories should be equipped with equipment and operators in strict accordance with the "Code of Practice for Clinical Gene Amplification Laboratories."
15) When using this kit, it should be operated strictly in accordance with the instructions; the specimen processing and specimen addition steps must be performed in a biological safety cabinet or other basic protective facilities, follow the technical requirements of the clinical gene amplification laboratory.
16) Follow your institution's environmental waste procedures for proper disposal of used cartridges and unused reagents.

8. Specimen Collection and Storage

[Exudate of Skin lesion]

1. Collect the exudate of skin lesion specimen into the commercially validated transport medium.
2. Specimens should be refrigerated 2-8°C (36-46°F) or frozen (-20°C or lower) within one hour after collection. The swab specimens can be stored for 7 day at 20°C (-4°F) and long-term storage below -70°C (-94℉).
3. Thawing on ice prior to use and during sample processing.

[Serum]

1. Collect the whole blood into the commercially available plain tube
2. If serum in the plain tube is stored in a refrigerator at 2-8°C (36-46°F). For prolonged storage, it should be at below -20°C (-4°F).
3. Thawing on ice prior to use and during sample processing.

[Plasma]

1. Collect the whole blood into the commercially available tube and centrifuge blood to get plasma specimen.
2. If plasma in tube is stored in a refrigerator at 2-8°C (36-46°F). For prolonged storage, it should be at below -20°C (-4°F).
3. Thawing on ice prior to use and during sample processing.

[Whole blood]

1. Collect the whole blood into the commercially available tube
2. If whole blood in tube is stored in a refrigerator at 2-8°C (36-46°F). For prolonged storage, it should be at below -20°C (-4°F).
3. In the pre-sample processing step, add 200ul of PBS to 100ul of whole blood to perform Vortex 30sec
4. Thawing on ice prior to use and during sample processing.

[Nasopharyngeal swab]

1. Hold the nasopharyngeal swab close to the nasal septum slowly and deeply to the back of the nasopharynx.
2. Rotate it several times to obtain secretions.
3. Quickly dip the swab into the specimen collection tube, and discard the tail.
4. Tighten the tube cap to seal in case of drying.
5. The swab specimens can be 1day at room temperature, 4 days at 2~8°C (36~46°F), and long-term storage below -70°C (-4°F).

[Oropharyngeal swab]

1. Use moderate swab to wipe the posterior wall of the pharynx and the tonsils on both sides avoiding touching the tongue.
2. Quickly dip the swab into the specimen collection tube, and discard the tail.
3. Tighten the tube cap to seal in case of drying.
4. The swab specimens can be 1day at room temperature, 4 days at 2~8°C (36~46°F), and long-term storage below -70°C (-4°F).

9. Procedure

Starting STANDARD M10 system

Note	For the detailed instructions, refer to STANDARD M10 system User Manual. If you have scanned the cartridge barcode in STANDARD M10 and the software version is not compatible, a 'Not Supported Device' error message appears. Update the software before proceeding the test.

1) Turn on STANDARD M10 system.
2) Check STANDARD M10 Console and STANDARD M10 Module is connected and functional.
3) Enter the User ID and Password on the Log In screen of STANDARD M10 Console and click the Log In button.

Figure 2. Log In screen

4) Touch STANDARD M10 Module to run on the Home screen.
(The door of the selected STANDARD ™ M10 Module will automatically open for cartridge loading.)

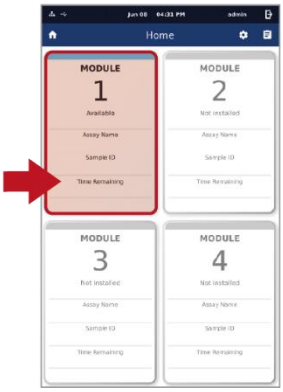

Figure 3. Home screen

5) Enter a Patient ID by scanning the barcode or using virtual keyboard on M10 Console screen.
(Patient ID is optional. You can turn off the Patient ID option from the 'Settings'.)

Figure 4. Entering Patient ID

6) **Enter a Sample ID by scanning the barcode of the specimen or using virtual keyboard on the M10 Console screen.**
Make sure that the specimen tube cap is firmly closed when scan the ID barcode printed on the specimen tube.
(For quality control test, tick the QC check box).

Figure 5. Entering Sample ID

7) **Scan STANDARD M10 MPX/OPX cartridge to be used. STANDARD M10 Console automatically recognizes the assay to be run based on the cartridge barcode.**

Figure 6. Scanning a cartridge

Loading a sample into STANDARD M10 MPX/OPX cartridge

caution	If the cartridge has been refrigerated, perform the test after stabilizing it for 30 minutes at room temperature (20-28°C, 68~82℉). Start the test within 10 minutes of loading the sample into STANDARD M10 MPX/OPX cartridge.
Note	False negative results may occur if insufficient sample is added into the cartridge.

1) Remove the Safety Clip located underneath the lid of the cartridge.
2) Pierce the sealed cartridge by pressing down the lid until fully engaged into the cartridge groove.
3) Open the lid and check that the seal is completely punctured before loading a sample.
4) Mix sample by rapidly inverting the specimen or external control tube 5 times. Carefully open the cap of the specimen tube or external control.
5) Dispense 300*µl* of the sample into the hole in the lower right corner of the cartridge using 300*µl* of disposal dropper or a pipette with a filter tip.
6) After a few seconds, Sample Guide screen will automatically change to the Insert Cartridge screen. Touch the Sample Guide screen if you want to skip the guide.

Figure 7. Sample Guide screen

7) Close the lid.

Figure 8. Loading a sample

Running a test

1) Load the cartridge on the selected STANDARD M10 Module with the Amplification chamber facing the inside of the module.
(The status indicator of the selected module will blink green.)

Figure 9. Insert Cartridge screen

2) **Close the door completely.**
3) **After confirm the sample and cartridge information, touch the OK button on the screen. (Touch the Reset button to re-input the information.)**

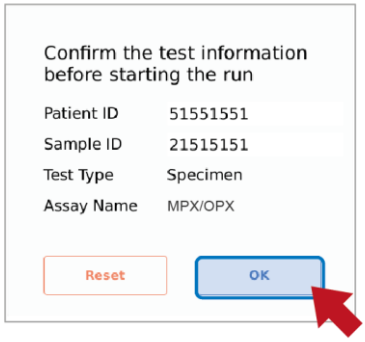

Figure 10. Confirm the test screen

4) **Assay starts automatically, and remaining time will appear on the screen.**

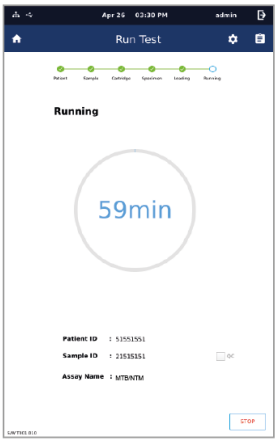

Figure 11. Running screen

5) **When the run is finished, it switches to the Review screen and the result is displayed.**
6) **Dispose of used cartridges in the appropriate sample waste containers according to your institution's standard practices.**
7) **To run another test, touch the Home icon and repeat the process. (If another STANDARD M10 Module connected to STANDARD M10 Console is available, you can start a new test while another test is running.)**

10. Interpretation of Results

The results are interpreted automatically by STANDARD M10 Console and are clearly shown in the Review screen. STANDARD M10 MPX/OPX test provides test results based on the detection of targets according to the algorithms shown in Table 3.

Table 3. Interpretation of results

Interpretation	OPX	MPX	IC
OPX Positive	+	-	+/-
MPX Positive	+	+	+/-
Negative	-	-	+
Invalid	-	+	+/-
	-	-	-
Error	No result		

Result	Expiations
OPX Positive	OPX (excluding MPX) viral DNA is detected. • The OPX signal has a Ct within the valid range. • The MPX signal does not have a Ct within the valid range. • IC: N/A (not applicable); IC is ignored because each target amplification occurred.
MPX Positive	MPX viral DNA is detected. • The OPX signal has a Ct within the valid range. • The MPX signal has a Ct within the valid range. • IC: N/A (not applicable); IC is ignored because each target amplification occurred.
Invalid	The OPX signal does not have a Ct within valid range. Presence or absence of MPX viral DNA cannot be determined since MPX belongs to OPX. Repeat test. • IC: Valid or Invalid; MPX and OPX viral DNA signals do not have a Ct within valid range. Presence or absence of OPX and MPX viral DNA cannot be determined. IC does not meet acceptance criteria. Presence or absence of MPX and OPX viral DNA cannot be determined. Repeat test. • IC: Invalid; IC, MPX and OPX viral DNA signals do not have a Ct within valid range.
Error	Presence or absence of MPX viral DNA cannot be determined. Repeat test. • Target : no result • IC: no result

11. Quality Control

STANDARD Quality Control procedures are intended to monitor cartridge and assay performance. If the controls are not valid, the patient results cannot be interpreted.

Internal control (IC): Ensures a proper sample has been applied, reagents in the cartridge are well functioning, there were no other interfering factors in the sample, and the procedure was performed correctly. In clinical samples showing positive signal for Monkeypox virus, the IC is reluctant and is ignored. If the IC fails where no Monkeypox virus are detected the result is invalid.

External controls should be used in accordance with local, state, and federal accrediting organizations as applicable.

12. Limitations

1) **Performance characteristics of this test have been established with the specimen types listed in the Intended Use Section only. The performance of this assay with other specimen types or samples has not been evaluated.**
2) **A false negative result may occur if :**
   - **Sample concentrations is near or below the limit of detection of the test**
   - **A specimen is improperly collected, transported or handled**

- Inadequate numbers of organisms are present in the specimen
- Cartridges are exposed to improper environmental factors (temperature / humidity)

3) False positive results may happen from cross-contamination between patient samples, specimen mix-up and/or DNA contamination during product handling.
4) Qualitative detection of positive results in this kit does not indicate the presence of live virus. It is recommended to use other methods for confirmation at the same time.
5) This kit only detects Orthopoxvirus and identifies Monkeypox virus. The test results are for clinical reference only. The clinical diagnosis and treatment of patients should be combined with their symptoms / signs, medical history, other laboratory tests and treatment responses considering.
6) Potential mutations within the target regions covered by the primer and/or probes of the test may result in failure to detect the presence of the pathogen.

13. References

1) Li, Y., Zhao, H., Wilkins, K., Hughes, C., & Damon, I. K. (2010). Real-time PCR assays for the specific detection of monkeypox virus West African and Congo Basin strain DNA. Journal of Virological Methods, 169(1), 223–227. doi:10.1016/j.jviromet.2010.07.012
2) Li, Y., Olson, V. A., Laue, T., Laker, M. T., & Damon, I. K. (2006). Detection of monkeypox virus with real-time PCR assays. Journal of Clinical Virology, 36(3), 194–203. doi:10.1016/j.jcv.2006.03.012
3) Li, D., Wilkins, K., McCollum, A. M., Osadebe, L., Kabamba, J., Nguete, B., Reynolds, M. G. (2016). Evaluation of the GeneXpert for Human Monkeypox Diagnosis. The American Journal of Tropical Medicine and Hygiene, 96(2), 405–410. doi:10.4269/ajtmh.16-0567
4) Alakunle, E., Moens, U., Nchinda, G., & Okeke, M. I. (2020). Monkeypox Virus in Nigeria: Infection Biology, Epidemiology, and Evolution. Viruses, 12(11), 1257. doi:10.3390/v12111257
5) WHO Interim guidance "Laboratory testing for the monkeypox virus" 23 May 2022

14. Symbols

REF	Reference number	LOT	Batch code
	Consult Instructions for Use		Manufacturer
	Contains Sufficient for <n> Tests		Date of manufacture
	Caution		keep dry
	Note		Keep away from sunlight
	Do not re-use.		Do not use if packaging is damaged
	Temperature limit		Use-by date
RUO	Research Use Only		