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Truenat™

Universal Control Kit

Control Kit for Truenat™ chip based micro PCR Tests

1. INTENDED USE

Truenat™ Universal Control Kit (REF 601100008) is a set of positive and negative control for validating the performance of **Truenat™** chip based micro PCR tests run on the **Truelab™ Uno** and **Truelab™ Uno Dx** Real Time micro PCR Analyzer.

2. INTRODUCTION

Testing for infectious diseases by detecting the pathogens nucleic acids using nucleic acid amplification methods is a highly specific and sensitive diagnostic tool. Molbio's **Truelab™** micro PCR System is a nucleic acid amplification platform that works on real time Polymerase Chain Reaction (PCR) technology that enables near patient diagnosis through **Truenat™** disposable, disease specific micro PCR chips and a portable, automated **Truelab™ Uno and Truelab™ Uno Dx** Real Time micro PCR Analyzer**.**

To ensure that the **Truenat™** chip based micro PCR tests and the **Truelab™** Real Time micro PCR Analyzer are working accurately, it is necessary to run positive and negative controls from time to time. The **Truenat™** Universal Control kit is a set of 1. Dried down mixture of calibrated Positive Control DNA (representing target nucleic acids of all **Truenat™** tests) and 2. Negative Control, that are run in place of nucleic acids extracted from specimen.

It is advisable to run controls under the following circumstances: • Whenever a new shipment of test kits is received. • When opening a new test kit lot. • If the temperature of the storage area falls outside of 2-30° C. • By each new user prior to performing testing on clinical specimen.

The Truelab™ Real Time micro PCR Analyzer is protected by the following patents and patents pending: IN 2313/CHE/2007, WO 2009/047804 and corresponding claims of any foreign counterpart(s) thereof.

The Truenat™ micro PCR chip is protected by the following patents and patents pending: IN 2312/CHE/2007, WO 2009/047805 and corresponding claims of any foreign counterpart(s) thereof.

3. PRINCIPLE OF THE TEST

Truenat™ chip based PCR tests work on the principle of Real Time Polymerase Chain Reaction. Six (6) µL of the reconstituted positive control or the negative control is pipetted into the reaction well of the **Truenat™** chip based micro PCR test. The **Truenat™** chip is then inserted in the **Truelab™** Real Time micro PCR Analyzer where thermal cycling takes place. A positive amplification causes the disease specific fluorescent probe in the **Truenat™** chip to release the fluorophore in an exponential manner which is then captured by the built-in opto-electronic sensor and displayed as amplification curve on the analyzer screen, on a real time basis during the test run. The Cycle threshold (Ct) is defined as the number of amplification cycles required for the fluorescent signal to cross the threshold (i.e. exceed the background signal). Ct value is linearly correlated with the initial load of target DNA present. The positive control in the **Truenat™** Universal Control Kit contains calibrated quantities of DNA representing target nucleic acids of all **Truenat™** tests to yield a range bound Ct value. This range is preset in the analyzer and the analyzer automatically compares this with the Ct value of the positive control. The negative control is a buffer solution which is also used to reconstitute the positive control, does not have any DNA and hence no amplification is expected to occur. In this case a horizontal amplification curve is displayed on the screen during the test run. The results screen on the analyzer displays "DETECTED" with Ct value or "NOT DETECTED" and whether the result is "Valid" or "Not valid". For a valid result, the positive control must amplify and the Ct value obtained should fall within the expected range. The negative control should not amplify. A repeatedly "Not Valid" result for positive control indicates a malfunction of either the **Truenat™** test or the **Truelab™** analyzer. Amplification ("Not Valid" result) for negative control indicates nucleic acid contamination in the testing place, equipments or reagents. An invalid result needs further investigation and resolution before carrying on any further testing. The results can be printed via Bluetooth using the **Truelab™** micro PCR printer or transferred to remote computer via Wifi network or 3G/GPRS network. The result is also stored in the analyzer memory for records.

4. CONTENTS OF THE Truenat™ UNIVERSAL CONTROL KIT

A. Individually sealed pouch, containing

1. A strip of 8 micro tubes each containing dried down Positive Control: 1 Strip.
2. A screw Cap vial containing 1 ml of Negative Control : 1 x 1 ml.

B. Package Insert.

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5. STORAGE AND STABILITY

Truenat™ Universal Control Kit is stable for one year from the date of manufacture if stored between 2-30°C. It is also stable for upto three (3) months at temperatures up to 40° C. Avoid exposure to light.

6. MATERIALS REQUIRED BUT NOT PROVIDED WITH THE KIT

Truelab™ Real Time micro PCR Workstation (REF 603010001/ 613010001/623010001) consisting of

1. **Trueprep™ MAG / AUTO** Sample Prep Device (REF 603040001/603041001).
2. **Truelab™Uno / Truelab™ Uno Dx** Real Time micro PCR Analyzer (REF 603020001/ 603021001**).**
3. **Truelab™** micro PCR Printer (REF 603050001).
4. **Truepet™** Precision Micropipettes.

Also required additionally are: **Truenat™** Chip Based micro PCR Tests, DNase and RNase-free pipette tips (2 - 200µL / 200 - 1000µL microtips) with filter barrier, which may also be procured from Molbio (REF 604072200 / REF 604062010 respectively), Powder free disposable gloves, waste disposal container with lid.

7. CONTROL PREPARATION

The dried down positive control in the micro tube needs to be reconstituted. Open the Zip Lock pouch, retrieve the positive control strip and cut out one positive control micro tube from the strip. Replace the remaining strip into the pouch and seal with the zip lock for future use. Pipette 50 µl of negative control from the negative control vial into the micro tube using a fresh DNase/ RNase free filter barrier micropipette tip. Discard the tip immediately into a waste disposal container. Mix the micro tube gently for a few minutes. The reconstituted positive control must be used immediately.

The negative control is a ready to use solution.

Caution: Positive control can cause contamination and should be handled with extreme care while opening the micro tube, reconstitution and use. Avoid spillage. Dispose and destroy the micro tube with left over control and micro pipette tips as described in section 13 below.

8. SAFETY PRECAUTIONS

1. For in vitro diagnostic use only.
2. Bring all reagents and specimen to room temperature (20 - 30^{0}C) before use.
3. Do not use kit beyond expiry date.
4. Carefully read the User Manuals and package inserts of Truelab™ Uno Real Time micro PCR Analyzer and Truenat™ chip based micro PCR test before use.
5. Do not pipette any material by mouth.
6. Do not eat, drink, smoke, apply cosmetics or handle contact lenses in the area where testing is done.
7. Use protective clothing and wear disposable gloves all through the testing process.

9. PROCEDURAL PRECAUTIONS

1. Check all packages before using the kit. Damage to the packaging does not prevent the contents of the kit from being used. However, if the outer packaging is damaged the user must confirm that individual components of the kit are intact before using them.
2. Do not perform the test in the presence of reactive vapours (e.g., from sodium hypochlorite, acids, alkalis or aldehydes) or dust.
3. While retrieving the Truenat™ chip based Real Time PCR test and the DNase & RNase free pipette tip from the pouch, ensure that neither bare hands nor gloves that have been used for previous tests run are used.

10. CLEANING AND DECONTAMINATION

1. Spills of potentially infectious material should be cleaned up immediately with absorbent paper tissue and the contaminated area should be decontaminated with disinfectants such as 0.5% freshly prepared sodium hypochlorite (10 times dilution of 5% sodium hypochlorite (household bleach) before continuing work.
2. Sodium hypochlorite should not be used on an acid-containing spill unless the spill-area is wiped dry first. Materials used to clean spills, including gloves, should be disposed off as potentially bio-hazardous waste e.g. in a biohazard waste container.

11. TEST PROCEDURE

(Please also refer the **Truelab™Uno /Truelab™ Uno Dx** Real Time micro PCR Analyzer user manual)

1. Switch on the **Truelab™** Analyzer.
2. If using the **Truelab™ Uno** device, also switch on the touch screen. If using the **Truelab™ Uno Dx** proceed to step 3.
3. Select user and enter password.
4. Select the test profile for the **Truenat™** chip based test being run, on the Analyzer screen.
5. Select Positive Control or Negative control as the case may be from the menu under **sample type** in the **Truelab™ Uno** Real Time micro PCR Analyzer screen. Ignore all other prompts on the screen.
6. Press Start Reaction.
7. Press the eject button to open the chip tray.
8. Open a pouch of **Truenat™** and retrieve the chip- based Real Time PCR test.
9. Label the chip as per control using a marker pen at the space provided on the back side of the chip.
10. Place the **Truenat™** chip on the chip tray without touching the white reaction well. The reaction well should be facing up and away from the Analyzer. Gently press the chip to ensure that it has seated in the chip tray properly.
11. Using the filter barrier tip provided in the pouch, pipette six (6) µL of the control into the centre of the white reaction well of the **Truenat™** micro PCR chip. Take care not to scratch the internal well surface and not to spill elute on the outside of the well.
12. Slide the chip tray containing the **Truenat™** chip-based Real Time PCR test loaded with the control into the **Truelab™** Analyzer .
13. Press Done on the "Please Load Sample" Alert message.
14. Read the result from the screen .
15. Take out the **Truenat™** micro PCR chip at end of the test and dispose it off as per the section on "Disposal and Destruction" (Section 13).
16. Turn on **Truelab™** micro PCR printer and select print on the screen for printing out hard copy of the results. Test results are automatically stored and can be retrieved any time later. (Refer to **Truelab™** Analyzer manual).
17. Switch off the **Truelab™** Analyzer.

12. RESULTS & INTERPRETATIONS

An amplification curve is displayed on the **Truelab™ Uno** Real Time micro PCR Analyzer screen to indicate the progress of the test. The curve will take a steep, exponential path when the

fluorescence crosses the threshold value in case of amplification. The curve will remain horizontal throughout the test duration in case of no amplification. At the end of the test run, the results screen will display “DETECTED” for Positive result or “NOT DETECTED” for Negative result. The result screen would also display the Ct value if amplification was detected. The result screen also displays the validity of the test run as “VALID” or “INVALID”. In the case of positive control, a valid result indicates that the control amplified and the Ct value was within the expected range. For negative control, a valid result indicates that there was no amplification detected. Invalid tests have to be repeated with fresh control and if still invalid for positive control, this indicates either that the lot of **Truenat™** test is not working or that there is a problem with the **Truelab™** analyzer. Check again with a new lot of **Truenat™**. If problem persists then contact **Molbio** support. Repeated invalid for negative control indicates contamination of the work area, the chip tray of **Truelab™** analyzer, pipettes, or the negative control itself. Check with a fresh vial of negative control. If not corrected, decontaminate work area, the chip tray of **Truelab™** analyzer and pipette using a wipe wetted with 0.5 % sodium hypochlorite and subsequently with clean water wipe and check with negative control. Repeat this procedure till problem is resolved. If problem persists then contact Molbio support.

Caution: Sodium hypochlorite can cause PCR reactions to fail. Ensure that the work area, surfaces and equipment are free of sodium hypochlorite or its vapor and confirm with positive control after every cleaning routine before proceeding with another test.

13. DISPOSAL AND DESTRUCTION

1. Submerge the used **Truenat™** micro PCR chip in freshly prepared 0.5% sodium hypochlorite solution for 30 minutes before disposal as per the standard medical waste disposal guidelines.
2. Disinfect the solutions and/or solid waste containing biological samples before discarding them according to local regulations.
3. Samples and reagents of human and animal origin, as well as contaminated materials, disposables, neutralized acids and other waste materials must be discarded according to local regulations after decontamination by immersion in a freshly prepared 0.5% of sodium hypochlorite for 30 minutes (1 volume of 5% sodium hypochlorite for 10 volumes of contaminated fluid or water).
4. Do not autoclave materials or solutions containing sodium hypochlorite.
5. Chemicals should be handled in accordance with Good Laboratory Practice and disposed off according to the local regulations.

SYMBOL KEYS

Consult instructions for use	IVD *In vitro* Diagnostic Test. Not for medicinal use.	Temperature Limitation	REF Catalogue Number	For single use only
Manufacturer	Date of Manufacture	Date of Expiry	LOT Batch Number / Lot Number	Σ Contains sufficient for <n> tests
EC REP Authorised Representative in the European Community				

Molbio Diagnostics Pvt. Ltd.

Registered Office:
H.No. 13, Sagar Society, Dona Paula
Panaji, North Goa,Goa - 403 722, India.
www.molbiodiagnostics.com

Manufacturing Unit:
Plot Nos. M 46-47, Phase III B,
Verna Industrial Estate, Verna,
Goa - 403 722, India.
Email: sales@molbiodiagnostics.com

EC REP
Qarad b.v.b.a. Cipalstraat 3, B-2440 Geel, Belgium

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