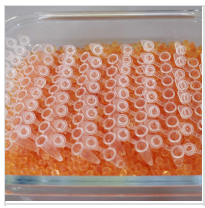

Version 2

Jul 05, 2022

Functionality test (OpenVent polymerase, PCR Master Mixes) V.2

In 1 collection

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This protocol is published without a DOI.

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ABSTRACT

After production, we typically subject our products to a batch of quality control assays to ascertain their functionality, efficacy and ability to meet their intended purpose.

Strict quality control is necessary when producing or using enzymes (whether purified or unpurified) and other molecular biology reagents.

Quality control for functionality involves incubating the enzymes or PCR master mix in a PCR reaction mixtures and testing for ability to amplify a specific region of a DNA template and the results recorded in terms of the presence or absence of amplification by bands on an electrophoresis agarose gel.

The Quality control tests are done at specific regular intervals and results documented for follow up. Incase any product fails the quality control test, actions are taken to verify he cause and make sure it does not repeat.

More than one test may be carried out for a particular product and the quality manager must approve and sign the certificate of analysis (a document that reveals the different quality control tests that have been performed on a reagent) to show that the product passes all the quality control tests assigned for it before a product is released for use.
Quality control of a product is done in two stages - the first involves a batch of tests carried out within the facility where the reagents are produced (Internal quality control) and the second involves testing for functionality by an external facility (External quality control).

In this protocol we describe how to carry out Functionality test of the following:

- OpenVent Enzyme
- PCR Master Mix - All formulations (wet and dry).

PROTOCOL CITATION

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COLLECTIONS

Beneficial Bio: Quality control tests

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PARENT PROTOCOLS

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Beneficial Bio: Quality control tests

GUIDELINES

Before carrying out quality control on products research should be carried out on the possible assays that apply for that particular product and availability of resources.

MATERIALS TEXT

The materials required for a quality control assays would depend on the products to be tested (whether Enzyme or PCR Master mix).

Reagents

- DNA polymerase of interest (either cellular reagent or purified enzyme or PCR master mixes)
- Control DNA polymerase (known to work)
- Forward Primer (20 pMol)
- Reverse Primer(20 pMol)
- PCR grade water
- Enzyme reaction buffer
- dNTP Mix (if necessary)
- Agarose (electrophoresis grade)
- TBE running buffer

Materials/Equipment

- Ice
- PCR machine (mini PCR-blueGel)
- Pipettes (P-10, P-20 and P-200)
- Sterile Pipette tips (10µl and 20µl)
- Bowl
- Sterile 0.2ml PCR tubes
- Electrophoretic gel tank and components(blueGel)
- Waste container

SAFETY WARNINGS

Wear protective clothing and all recommended Laboratory PPE to avoid contaminating the reagents as this may affect their functionality.
PPE may also protect you from accidental spills or splashes that may be dangerous to the eye or skin.

BEFORE STARTING

Clean and disinfect all work surfaces with a 1:10 dilution of bleach followed by 70% alcohol.

Functionality test

1 DNA polymerase Enzyme and PCR Master mix

After establishing a successful protein expression through SDS PAGE using the method described in this protocol, functionality test is performed.

In this section we describe the procedure for testing Enzymes (DNA polymerases - OpenVent) and PCR Master Mix.

- We adopt **20 µL** PCR reaction in our lab, **25 µL** and/or **50 µL** reactions are can be made but do not forget to calculate the corresponding amount of PCR reagents.

Pipetting:

1. Thaw all reagents on ice in a bowl
2. Label reaction tubes (PCR tubes) according to the number of samples, and including controls in each run (negative and positive controls) as needed.

Polymerase enzyme type:

1. If using a 10x cellular reagent enzyme- Rehydrate the enzyme with 30µlof sterile PCR grade water, flick tube and keep on ice
2. If using a pre-purified polymerase or Master mix remove from the freezer and keep on ice.

In each PCR tube, pipette and combine the following reagent components following the order in the table below *(work on ice while pipetting*).

A	B	C	D	E
PCR component	**Test sample-1x PCR Master Mix**	**Test sample-DNA polymerase enzyme**	**Negative control (for OpenVent Enzyme)**	**Positive control (for OpenVent Enzyme)**
PCR water	Variable to 20ul	Variable to 20ul	Variable to 20ul	Variable to 20ul
dNTP(10mM)	/	0.4ul	0.4ul	0.4ul
PCR buffer(10x)	/	2ul	2ul	2ul
Forward primer	1ul	1ul	1ul	1ul
Reverse primer	1ul	1ul	1ul	1ul
DNA template	1ul	0.5-1ul	/	0.5ul-1ul
Test enzyme	17ul	1ul	1ul	/
Control Enzyme (Commercial DNA polymerase)	/	/	/	1ul

1. The negative control is done by pipetting all PCR components without DNA template to ensure there were no pipetting errors because no DNA amplification band is expected after agarose gel electrophoresis.
2. The positive control is done by pipetting all PCR components with a commercial DNA

polymerase enzyme as a standard to ensure thermocycling process was successful because amplification of the DNA template is expected.

3. The control parameters represented in the table are for OpenVent Enzyme, for the PCR Master Mix we use same principle pipetting appropriate amount of reagents that correspond to Master Mix.

2 Thermocycling:

1. Connect a Thermocycler and program the protocol to run following the thermocycling steps listed below. The exact annealing temperature will depend on the choice of DNA template and primers used.

Initial Denaturation	95	120 sec
Denature	95°C	30 secs
Anneal	50-65°C(depending on primers)	20 sec
Extend	72°C	Variable based on primers
Final Extension	72°C	120secs

- The time of run will depend on the size of the DNA template and number of cycles of amplification (usually we use do 35 to 40 cycles for 0.5kb Lambda-50ng/ul).
- At the end of the thermocycling process, run agarose gel electrophoresis to check for amplification of the DNA template.

3 Preparing Agarose gel for Electrophoresis

Follow this protocol to prepare and cast the gel.

4 Loading the gel wells

Pipette 3 to 5µl of DNA ladder (size depending on size of the DNA template) unto the first well then pipette the negative control carefully to avoid bubbles and load into the second well.

Follow the step in loading by pipetting 1part of the DNA loading dye and 5 parts of amplicon or test sample, mix your amplicons with DNA loading dye and load in the subsequent wells (in the case of the master mixes, they are loaded directly as they already contain the tracking dye and components to help them sink in the well).

Allow the gel to run for 15-30mins at 48V on 0.8, .5% or 2% agarose gel in TBE buffer.

Visualization of gel and Interpretation of gel results

The amplification results are visualized by transferring the gel into a UV transilluminator or blue light transilluminators.

With the help of the DNA ladder, determine if the amplification product is of the expected size and the bands are sharp and distinct looking exactly like those from the DNA polymerase enzyme and PCR Master Mix standards.

If no bands are seen at the expected band size at the lanes of the test sample, it means there was no amplification hence the test reagent is not working (if bands are seen at the positive control).
The results are recorded for further actions.