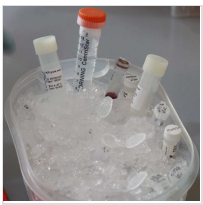

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Nuclease Test (OpenVent polymerase, PCR Master Mix, DNA loading dye)

In 1 collection

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1 *Works for me* Share

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ABSTRACT

Quality control involves incubating the enzymes or reagents in reconstituted recombination assays to eliminate the possibility of relevant protein or nucleic acid contaminants that may interfere with recombination assays and lead to misinterpretation of experimental data. Here batch by batch quality controls are done for each new product before it is released to the market.

This protocol describes the steps in carrying out Nuclease test for:

- OpenVent Enzyme
- PCR Master Mixes - All formulations (Wet and Dry)
- 6x DNA loading dye

PROTOCOL CITATION

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COLLECTIONS

Beneficial Bio: Quality control tests

KEYWORDS

Quality control of Recombinant protein, Quality control of DNA polymerase enzyme, Nuclease activity

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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. This protocol describes how to check Nuclease activity for PCR Enzymes, PCR Master Mix and DNA loading dye.

MATERIALS TEXT

Chemicals

- Agarose
- Enzyme of interest
- Enzyme specific buffer
- Nuclease free water
- Purified plasmid DNA
- DNase1-0.1g/ml (an endonuclease to act as positive control that digests double- and single-stranded DNA into oligo- and mono-nucleotides). 1mg/ml DNase1 can be used for optimal results. So up to 2µl can be used.
- DNA loading dye
- TBE running buffer

Materials and Equipment

- 0.2ml PCR tubes
- 37°C water bath
- Horizontal gel tank including trays and power pack
- UV/blue light transilluminator
- Image recording device like a phone or camera

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.
Make sure all resources needed are prepared.

Nuclease activity

1

The Nuclease test will vary in components depending on the product whether it is the enzyme or PCR master mix. The Enzyme buffer is not added when testing PCR Master Mix.

The incubation times may vary depending on the product being tested. We typically use 4 hours for Enzymes and 16 to 20 hours for other products(DNA loading dye).

Summarily the Nuclease test involves incubation of Reaction Buffer containing a minimum of 20 units of Enzyme (test enzyme/ PCR master mix) with 1 µg of pOBL1 or PUC19 plasmid DNA-50pg/ul for 4 hours at 37°C results determined by agarose gel electrophoresis 1.5% (w/v) agarose gel with TBE buffer system.
In our lab we use pOBL1 plasmid but any circular double stranded DNA can be used.

2 **Pipetting**

Pipette the following reagents into 0.2ml reaction tubes as shown in the table below, while working on ice

A	B	C	D	E	F
Component	**Test Sample (Enzyme and PCR Master mix)**	**Negative control**	**Positive control**	**Test Sample (DNA loading dye)**	
Enzyme specific buffer	2µl	2µl	2µl	2µl	
Test sample	5µg	/	/	5µg	
Plasmid DNA	1µg	1µg	1µg	1µg	
DNase1	/	/	2units (0.5-2µl)	/	
PCR water	Variable to 20µl	Variabke to 20µl	Variable to 20µl	Variable to 20µl	
Incubation time	4 hours	Same time as test sample	Same time as test sample	14 to 16 hours	

Incubate reaction tubes for the specified times and briefly place tubes on ice to stop the reaction.

Checking and interpreting results:

After incubation period the reaction is stopped, and a1.5% agarose gel prepared, run and visualized as in this protocol.

Any change in band intensity and migration position of the plasmid DNA bands compared to the negative control signals potential endonuclease activity. Linear DNA should run true to size(known size of plasmid used), supercoiled DNA should run faster and relaxed circle DNA should run slower(a single strand cut).