Version 2

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Nuclease Test (OpenVent polymerase, PCR Master Mix, DNA loading dye) V.2

In 1 collection

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ABSTRACT

Quality control and nuclease test 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

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

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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
- Water bath
- Horizontal gel tank including gel casting tray, well combs 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 (Lab coat, gloves, face mask) 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 50m

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 the 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 a reaction mixture containing a minimum of 20 units of enzyme (test enzyme/ PCR master mix) with 1 µg of pOBL1 or PUC19 plasmid DNA-50 pg/uL for 4 hours at 37°C.

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The nuclease activity of the test product is determined by visualizing the migration pattern of the DNA plasmid on 1.5% (w/v) agarose gel electrophoresis.
In our lab we use pOBL1 plasmid but any circular double stranded DNA can be used.

2  15m

Pipetting

- Pipette the following reagents into 0.2 mL PCR 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	2 µl	2 µl	2 µl	2 µl	
Test sample	5 µg	/	/	5 µg	
Plasmid DNA	1 µg	1 µg	1 µg	1 µg	
DNase1	/	/	2 units (0.5-2 µl)	/	
PCR water	Variable to 20 µl	Variable to 20 µl	Variable to 20 µl	Variable to 20 µl	
Incubation time	4 hours	Same time as test sample	Same time as test sample	14 to 16 hours	

The negative control comprises all reaction components without the DNase 1. *The plasmid DNA should still maintain its circular conformation here.*
The positive control comprises all reaction components with DNase 1 *(enzyme endonuclease that nonspecifically cleaves DNA and make it to take a linear or relaxed conformation)* – to be used as "reference" or "standard" to compare the test sample.

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Incubation

- Incubate reaction tubes for the specified durations.
- After the incubation period, remove the tubes from the water bath and briefly place them on ice to stop any further the reaction.

The incubation period would depend on the test sample (For example, we adopt 4 hours for enzyme and PCR master mixes and 16 hours for DNA loading dye).

4 

35m

Checking and interpreting results:

- After the incubation period, prepare a 1.5% agarose gel, run and visualized as described in this protocol.
- Supercoiled DNA should run faster (as would be expected in the negative control lane). Meanwhile, linear DNA should run true to size (known size of the plasmid used), and relaxed circle DNA should run slower in a single strand cut (as would be expected in the positive control lane).
- Any change in the band intensity and migration position of the plasmid DNA band in the test sample lane compared to that of the negative control signals potential endonuclease activity of the test sample.

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