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IdentityPlant 2X Master Mix for direct PCR from plants

Direct PCR amplification from plant parts (leaves, stem, roots)

Cat#: M3086

Version: 200313

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- Chemicals
- Biochemicals
- Cell Culture Products
- Antibodies and Cytokines
- Molecular Biology Products
- PCR
- Proteins and Enzymes
- Consumables

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Related Products

	Contents	Cat. No.
SNP Pol DNA Polymerase For SNP analytics – direct detection of point mutations without the need for sequencing	250 units 1000 units	M3009.0250 M3009.1000
Taq-S DNA Polymerase high efficiency	250 units 1000 units 2500 units	M3001.0250 M3001.1000 M30001.2500
Taq-E DNA Polymerase high efficiency	250 units 1000 units 2500 units	M3043.0250 M3043.1000 M3043.2500
SuperHot DNA Polymerase Hotstart DNA polymerase	250 units 1000 units	M3307.0250 M3307.1000
ProbeMasterMix 2X- No ROX 2-time master mix for qPCR with Probes	2 x 1.25mL 10 x 1.25mL	M3045.0100 M3045.0500
GreenMasterMix 2X- No ROX 2-time master mix for qPCR with Green DNA dye	2 x 1.25mL 10 x 1.25mL	M3023.0100 M3023.0500
RedMastermix 2X master mix for standard PCR including a red tracking dye	2 x 1.25mL 10 x 1.25mL	M3029.0100 M3029.0500
GenaxxoFast qPCR Master Mix Mastermix for Fast PCR	1mL 5 x 1mL	M3067.0100 M3067.0500
IdentityTaq 2X master mix for direct PCR from food of animal origin	2 x 1.25mL 10 x 1.25mL	M3089.0100 M3089.0500
Random hexamer primer lyophilized primer	5 OD	M3038.0125
dNTP-Set (100mM) nucleotides for PCR	4 x 20µmol 4 x 25µmol 4 x 100µmol	M3015.4020 M3015.4025 M3015.4100
dNTP-Mix (10mM) nucleotides for PCR	200µL 1000µL	M3016.0200 M3016.1010

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IdentityPlant 2X Master Mix for direct PCR from plants

Direct PCR amplification from plant parts (leaves, stem, roots)

Product	Cat#	Package size
IdentityPlant 2X Master Mix	M3086.0100	1mL (of master mix); 22mL rapid lysis buffer
IdentityPlant 2X Master Mix	M3086.0500	5 x 1mL (of master mix); 5 x 22mL rapid lysis buffer

Product Description

The Genaxxon IdentityPlant 2X master mix in combination with the special lysis buffer for Direct PCR is an optimized ready-to-use mixture, suitable for plant species validation, genotyping, GMO testing or plant pathogen identification. It contains a modified fast HotStart Taq DNA Polymerase, dNTPs and $MgCl_2$ and all other components required for PCR except primers and template DNA. Most importantly, the IdentityPlant 2X master mix has a high tolerance to many plant-derived PCR inhibitors such as polyphenolics and polysaccharides.

Small amounts of crude plant extracts from i.e. leaves, stem, roots, seeds or other plant tissues, prepared with the special lysis buffer, can directly be used for PCR. PCR assays with the IdentityPlant 2X master mix not only reduce contamination risks, but are also time-saving, highly reproducible, easy to prepare and show a high tolerance to PCR inhibitors.

Due to the optimized composition of the IdentityPlant 2X master mix, the HotStart Taq polymerase delivers specific PCR amplification of good yield. The IdentityPlant 2X master mix is able to amplify PCR products up to at least 3 kb. The Taq polymerase included in the master mix possesses a 5´→ 3´ polymerase- as well as a 5´-flap endonuclease activity and generates a 3'dA (adenine)-overhang.

The lysis can be performed in a thermocycler or in a heating block at two temperature steps, one at 55°C for 10 to 15 minutes and the second at 98°C for 15 minutes. After the lysis step the sample is centrifuged to recover the DNA containing supernatant which can be used without further dilution.

Unlike some protocols using Proteinase K digestion and separate extraction of DNA, the IdentityPlant 2X master mix lysis extracts do not need any separate extractions with toxic or hazardous chemicals which reduces the risk of sample loss and contamination. Each extraction yields a sufficient amount of template for up to 20 genotyping PCR reactions. Extracts are stable for at least 6 months at -20°C.

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Applications

IdentityPlant 2X master mix is recommended for use in Direct PCR applications for plant species validation, genotyping, GMO testing or plant pathogen identification. Small amounts of crude plant extracts from i.e. leafs, seeds or other plant tissues, prepared with our RTL buffer, can directly be used for PCR. The IdentityPlant 2X master mix is able to amplify PCR products up to at least 3 kb.

Contents

IdentityPlant 2X master mix is containing all the components necessary for direct PCR, including an optimized reaction buffer, ultrapure dNTPs (M3015), a specifically engineered HotStart DNA polymerase and an optimized lysis buffer.

Provided reagents:

Sample-Lysis Buffer (22mL).
Ready-to-use 2X Master Mix, 1mL (80 x 25µL PCR reactions).

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Unit definition

One unit of the IdentityPlant PCR DNA polymerase is defined as the amount of enzyme that incorporates 10nmol of dNTPs into acid-insoluble fraction in 30 minutes at 72°C under standard assay conditions.

Quality Control

DNA polymerase activity: IdentityPlant PCR DNA polymerase activity is monitored and adjusted to a specific DNA polymerase activity using an artificial DNA template and a DNA primer.

PCR activity: Functionality of the Genaxxon bioscience IdentityPlant 2X master mix is tested for successful PCR performance by amplification of a 400bp and a 1kb amplicon. The PCR result is analyzed by agarose gel electrophoresis.

Impurity test: The IdentityPlant 2x master mix is tested to show negative results in negative controls (no animal DNA in the IdentityPlant preparation).

Enzyme concentration is determined by protein-specific staining. Please inquire more information at info@genaxxon.com for the lot-specific concentration.

Stability

Genaxxon bioscience IdentityPlant 2X master mix is shipped on wet ice and should be stored immediately upon receipt at -20°C. Minimize the number of freeze-thaw cycles by storing in aliquots. For day-to-day use, we recommend keeping an aliquot at +2°C to +8°C.
When stored under these conditions and handled correctly, these products can be kept at least until the expiration date (see tube label) without showing any reduction in performance.

Safety information

This product does not require a Material Safety Data Sheet because it does neither contain more than 1% of a component classified as dangerous or hazardous nor more than 0.1% of a component classified as carcinogenic. However, we generally recommend, when working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.

Genaxxon bioscience takes no liability for damage resulting from handling or contact with this product.

More information can be found in the REGULATION (EC) No. 1272/2008 OF THE EUROPEAN PARLIAMENT AND THE COUNCIL or contact Genaxxon bioscience (info@genaxxon.com)

This product is for research use only

Tips and tricks

Use gradient PCR to find optimal annealing temperature

By using the specially developed Hotstart Taq polymerase and the special lysis buffer in the IdentityPlant 2X master mix, it is very likely that the optimal annealing temperature in PCR differs significantly from the usual conditions. To find the optimal annealing temperature, a gradient PCR should be run first.

Small amounts of starting material

If there is little starting material and the PCR results are weak, the following tips may be helpful:

- reduce amount of lysis buffer from 200µL to 100µL
- use more lysate for PCR (4µL instead of 2µL)
- increase cycle number (up to 45)

Trouble shooting:

Comment - Cycling protocol

Due to the use of the specifically engineered DNA polymerase in the IdentityPlant 2X master mix, it is critical that the correct cycling conditions are used.

The unique lysis buffer system is most likely different to that used with Proteinase K digested tissue and subsequent standard PCR. For this, most likely annealing temperatures of the PCR protocol are different from that used without the IdentityPlant 2X master mix.

Please adopt annealing temperature by gradient PCR!

If you do not get satisfying results adjustments should be made as follows:

Low Yield:
- If a low yield of the specific product is obtained, lower annealing temperature in 2°C increments.

Nonspecific Product:
- If nonspecific products are formed in addition to the specific product, increase annealing temperature in 2°C increments.

No Product:
- If no specific product is formed reduce annealing temperature by 4°C.
- MgCl2 concentration may have to be increased.

Annealing temperature gradient PCR is a quick and easy method to determine the optimal annealing temperature for a mouse genotyping assay.

Important Parameters

DNA extraction (Lysis of sample)

IdentityPlant 2X master mix rapid tissue lysis buffer is capable of rapid digestion of tissue and cells in leaves, stem, roots, seeds or other plant tissues. Unlike Proteinase K, the IdentityTaq lysis buffer may not always degrade tissue completely. Visible remaining tissue in the extraction tube is no indication for a failure of the process.

Preparation of crude plant lysate

Components		Working steps
Lysis buffer	200µL	pipette into an appropriately sized reaction tube, i.e. 1.5ml
Plant sample	2x2 mm - 4x4 mm	add plant sample, submerged in the buffer
Water bath or heating block	At 56°C	incubate for 15 min
Water bath or heating block	At 95°C	incubate for 15 min
Benchtop centrifuge	≧ 15.000 rcf	place tube into centrifuge and centrifuge at full speed for 5 min
Reaction tube	i.e. 1.5 ml	- transfer 180 µl of the lysate supernatant into a new tube for storage at 4°C or -20°C - or use lysate directly for PCR

Primers

Primers should be designed to eliminate the possibility of primer-dimer formation and nonspecific annealing, and should have a GC content of 40-60%. Primers with a GC content >65% may require higher denaturation temperatures and/or longer denaturation times, while primers with a GC content <40% may require annealing temperatures <60°C, which is not recommended. For specificity reason, annealing temperatures above 60°C are highly recommended. A GC content of <40% may also need an increased MgCl2 and primer concentration.

Furthermore, primer sets should be designed to have similar theoretical melting temperatures.

NOTE: **It is mandatory to add a negative control to each PCR experiment!**

NOTE: Always dilute and store primers in a buffered solution (e.g. 10mM Tris/HCl, pH8.0-8.5) instead of PCR-grade water to limit degradation of primers.

PCR Set-up

Before starting

- Setup can be done conveniently at room temperature.
- Spin down and mix all solutions carefully before use.
- Primers should ideally have a GC content of 40% to 60%.
- Primers have to have a melting temperature of >60°C!
- Minimize the number of freeze-thaw cycles by storing in aliquots. For a day-to-day use, we recommend keeping an aliquot at +2°C to +8°C.

Table 2: Recommendations for PCR / Reaction Setup (20µL PCR reaction)

Components	Sample PCR	Negative control PCR	Sample concentration (recommended)
IdentityPlant 2X master mix	10µL	10µL	1x
Primer forward (e.g., 5pmol/µL)	variable (e.g., 1 µl)	variable (e.g., 1 µl)	0.1 – 0.5 µM
Primer reverse (e.g., 5pmol/µL)	variable (e.g., 1 µl)	variable (e.g., 1 µl)	0.1 – 0.5 µM
Template DNA	2µL lysate	---	---
Sterile distilled water	Adjust to 20 µl final volume	Adjust to 20 µl final volume	

Primers should ideally have a GC content of 40-60%.
Primers have to have an annealing temperature of >60°C!
Final primer concentrations can be in the range of 0.1-1µM, but we recommend starting with the given value of 0.4µM.

NOTE: In case PCR result with 2µL lysed sample extract is poor, try 3µL! Reduce amount of nuclease-free water accordingly.

Notes:

1. In some cases soft plant tissues may directly added to the PCR reaction without prior lysis buffer treatment. However, then samples should be 1x1mm in size or smaller.
2. For some plant samples, i.e. seeds, roots or conifer needles, it may be advisable to mechanically disrupt/homogenize the sample prior to lysis by cutting or mashing the sample.
3. Best PCR results will be obtained with lysate amounts of one-tenth of the total PCR volume. Do not exceed lysate amounts of one-fifth of your total PCR volume.

Table 3: Typical 3-step PCR protocol

Important: The IdentityPlant 2X master mix contains a specifically engineered HotStart DNA polymerase and a unique lysis buffer that require optimized reaction conditions. If these conditions are not adhered to, reaction failure is likely.

Step	Temperature	Time
Initial denaturation	92° - 95°C	2 min.
Denaturation	92° - 95°C	5 – 10 sec.
Annealing*	55°C - 68°C	2 - 10 sec.
Extension**	72°C	variable, depends on the length of product
Hold	<10°C	hold

* For maximum yield and specificity, annealing temperatures and annealing time as well as extension time and cycle numbers should be optimized for each template target and primer pair. Usually the optimal annealing temperature is 2-5 °C below the melting temperature of the primers.

** Elongation times of 30 secs. per 1 kb may be sufficient but longer elongation times may be necessary depending on the complexity of the template DNA.
Number of cycles: 30-45 cycles

NOTE: Buffer conditions are different to that used if DNA is extracted by Proteinase K. For this reason, annealing temperature using IdentityPlant 2X master mix are very likely different to the annealing temperature needed using Proteinase K and subsequent PCR

Program the thermal cycler according to the manufacturer's instructions.
A typical PCR cycling program is outlined in Table 3. For maximum yield and specificity, temperatures and cycling times should be optimized for each new target or primer pair.

Place PCR tubes in the thermal cycler and start program.