Fast Saliva Genotyping Kit

Product Information:

Kit contains: Saliva Genomic DNA Extraction Reagent-A (5ml); Reagent-B (500ul); Reagent-C (5ml); DNA Precipitation Solution (10ml); 2x PCR Mastermix (1.25 mL);
Catalog: GT-01Sp
Sizes: 100 extractions
Storage: -20ºC

Description:

1. The fast genotyping reagents contain a combination of enzyme(s), detergents, and PCR required reagents that lysate the mammal saliva/blood samples within 20 minutes and directly used to run PCR reactions without any further DNA purification.
2. 2x PCR Mastermix contains 2x PCR buffer, 2x loading buffer, heat-activated Taq DNA polymerase, dNTPs (dATP, dCTP, dGTP, dTTP), 3mM MgCl2, PCR buffer stabilizer and green tracking dyes. The PCR products are directly loaded onto agarose gels without adding the DNA loading buffer.
3. No filter column and toxic organic solvents are involved, able to reduce the loss of DNA during extraction procedures, suitable for nucleic acid extractions from the small amount of saliva/blood samples including frozen or fresh samples.
4. The purified saliva DNA is suitable for PCR, qPCR, southern blot, sequencing and other DNA assays.

Procedure:

1. Prepare 20-50ul saliva sample in a clean 0.5ml or 1.5ml microcentrifuge tube.
2. Add 50ul reagent-A and 5ul reagent-B into the sample tube. Incubate at 60ºC for 10 minutes.
3. Place the tube in a PCR machine (or water bath or block) and incubate at 95ºC for 10 minutes.
4. Centrifuge at 12,000 xg for 2 minutes at 4ºC and transfer the lysate supernatant into a clean tube.
5. Add 50ul reagent-C into the lysate supernatant and mix by vortexing.
6. Pipette 2-10ul lysates into a 25ul PCR Master Mixture and run PCR/ qPCR at thermal cyclers.

Suggested PCR Protocol:

I. Preparation of PCR Master Mix for a single reaction (total volume: 25uL) in a 0.2 or 0.5mL microtube.

Component	Volume (µL)	Final Concentration
Lysate supernatant	2-10	determined by user
2x PCR Mastermix	12.5	1x
Forward primer (5µM)	1	200nM
Reverse primer (5µM)	1	200nM
PCR grade water	up to 25 µL	

II. Setup typical thermal cycling parameters

Enzyme activation step:	95°C	5 minutes
25-40 cycles:		
Denaturation	95°C	30 seconds
Annealing	X°C	30 seconds dependent on Tm of primers
Extension	72°C	30 seconds (1min per kb amplicon)
Hold at 4-8°C Directly load PCR products onto agarose gels without adding loading buffers.		

Technical Tips:

A new technical procedure using DNA precipitation solution for obtaining high-quality DNA, prepared for PCR, qPCR, Microarray and Southern Blot assays

Procedure :

1. **Prepare 50ul saliva sample in a 1.5ml microcentrifuge tube.**
2. **Add 50ul reagent-A and 5ul reagent-B into the sample tube. Incubate at 60ºC for 10 minutes.**
3. **Place the tube in a PCR machine (or water bath or block) and incubate at 95ºC for 10 minutes.**
4. **Centrifuge at 12,000 xg for 2 minutes at 4ºC and transfer the lysate supernatant into a clean tube.**
5. **Add 50ul reagent-C into the lysate supernatant and mix by vortexing.**
6. **Centrifuge at 5,000 xg for 5 minutes at 4ºC and transfer the lysate supernatant into a clean tube.**
7. **Add 100ul 2x DNA precipitation solution (Cat.#: PS-01D) into the lysate supernatant.**
8. **Mix well and centrifuge at 12,000 xg for 10 minutes at 4ºC.**
9. **Aspirate liquid and simply rinse pellet with 80% ethanol for 2 times (don't resuspend the DNA pellets). The DNA pellets will not be visible if the concentration is less than 20ng/ul.**
10. **Air-dry DNA pellet for 5-10 minutes.**
11. **Dissolve the DNA pellets in 20 ul of nuclease-free H_2O or TE if the DNA pellet is visible. Otherwise, use 10ul of nuclease-free H_2O or TE.**
12. **Measure DNA concentration using a spectrometer and store DNA at -20°C or 2-8°C.**

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Figure:
PCR analysis of genomic DNA extracts from saliva and blood samples using Zmtch Fast Saliva genotyping kit (GT-01Sp). Genomic DNA in 5ul lysate supernatant was directly amplified in a 25 ul PCR reaction for 30 cycles.

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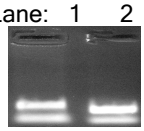

(Saliva Blood)

Precautions and Disclaimer:

This product and procedure described are intended for R&D use only. Purchase of this product does not convey a license to perform any patented process.

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