

PROTRANS Domino System HLA – disease associations Narcolepsy

REF 201 091

Instruction

- Take the Protrans PCR workstation out of the freezer (-20°C).
Take the Protrans **Buffer R (yellow cap)** and Protrans Buffer Y out of the freezer (-20°C), thaw it and place it in the PCR workstation.
- Take the Protrans **Narcolepsy Domino-Strip** out of the refrigerator (2-8°C) and place it in the PCR workstation.
- Preparation of the **Pre Master Mix**.
For each DNA sample pipette in a 1.5ml reaction tube:

Protrans SSP Cyclor Program			
Initial denaturation	94°C	2 min	Hold
Denaturation	94°C	15 sec	10 cycles
Annealing and Extension	65°C	60 sec	
Denaturation	94°C	15 sec	20 cycles
Annealing	61°C	50 sec	
Extension	72°C	30 sec	
Hold	4°C	15 min	Hold
Ramp rate 1°C/sec			

Protrans Domino-Strip Narcolepsy	Buffer R	Buffer Y	Taq Polymerase 5U/µl
	23 µl	46 µl	0.6 µl

- Vortex **Pre Master Mix** very thoroughly and spin the tube down.
 - Mark the Protrans Narcolepsy Domino-Strip.
 - Dispense **10µl** of the **Pre Master Mix** without the DNA in the **Negative Control - position 7**.
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- Add **20 µl of the DNA sample (50-100ng/µl)** to the **Pre Master Mix**.
 - Vortex **Master Mix** including the individual DNA very thoroughly and spin the tube shortly.
 - Dispense of each Master Mix** with a stepper:
10µl in all 6 positions of the Protrans **Narcolepsy Domino-Strip**.
 - Shake down the red Master Mix drops in the Protrans Narcolepsy Domino-Strip.
 - Close the Protrans Narcolepsy Domino-Strip with the cap strips and seal it very carefully by pressing the caps into the wells.
 - Place Protrans Narcolepsy Domino-Strip directly in the thermocycler, final volume 10µl, and start the amplification immediately or store the covered Domino-Strip at 2-8°C and start the amplification within 2 hours.

