Toxoplasma PCR detection Kit

For Research Use Only

Cat. No.: PK3141 **Quantity:** 50 Reactions
Shipment: Wet/Dry Ice **Storage:** -20°C

SinaClon Toxoplasma gondii PCR Detection kit is destined for qualitative detection of T.gondii DNA in infected samples by the method of Polymerase Chain Reaction. The reagent of ready to use mix is an optimized 1X PCR mixture of Taq DNA Polymerase (recombinant), PCR buffer, $MgCl_2$, dNTPs and primers. Primer set is specific to the highly specific repetitive region of B1 gene. This primer set, allows for detection of 45 copies of Toxoplasma gondii.

Kit Contents:

1. **1X PCR MIX**	1000 µl	4. **DNase Free, Deionized Steril Water**	2.0 ml
2. **Taq DNA polymerase**	10 µl (5u/µl)	5. **Mineral Oil**	1.0 ml
3. **Positive Control**	100 µl		

Sample preparation:
DNA Extraction: Performed in Pre-amplification 1, Specimen & Control Area.

DNA can be extracted by commercial stool and soil DNA extraction kits or in-house method.
Human or tissues samples can also be extracted by SinaClon DNA extraction kits: DNP™ (Cat. No.: EX6071) or SinaPure™ (Cat. No.: EX6011).

PCR Protocol:
Performed in Pre- Amplification 2, Reagent Preparation Area.

1. Take out the kit and unfreeze the tubes, then put all the tubes on ice. The final volume of each reaction will be 25ul.
2. Label new PCR tubes for amplification reaction(s) for test(s), positive and negative control.
3. Add the following reagents for each tube on ice:

PCR MIX	19.8 µl/ for each reaction
Taq DNA polymerase	0.2µl/ for each reaction

4. To each tube add one drop (20-25 µl) mineral oil (if needed). Cap the reactions tube or Place the tube tray in a reseal able plastic bag and seal the bag securely. Next steps should be done at:

Pre –Amplification 1 , Specimen & Control Preparation Area
5. Add 5 µl DNA*(Use specified pipette for sampling of DNA).
6. Close tubes; spin the mixtures on microfuge for 3-5 sec.
7. Transfer the tubes to preheated thermo cycler and start the program:

Cycling parameters:

Initial Denaturation:	94°C - 5 min	1x then link to
94°C – 30 sec 60°C - 30 sec 72^{0}C- 30 sec		34 Cycles
Final Extension	72^{0}C- 5 min	1x

(Cycling parameters may need to be setup with some Thermo cyclers.

Result analysis:

Performed in Post –Amplification Area.

In presence of 50bp DNA ladder, analyze 10µl of amplified samples directly in a 2.5% agarose gel without adding loading buffer. The presence of 115bp fragments indicates positive test.