Platinum® GenoTYPE *Tsp* DNA Polymerase

Cat. No. 11448-032
Conc.: 5 *Tsp* U/µl

Size: 2500 *Tsp* Units
Store at -20°C in a non-frost-free freezer.

Description: Platinum® GENOTYPE *Tsp* DNA Polymerase is a recombinant DNA polymerase from a thermophilic species of bacteria. It is intended for use in genotyping of dinucleotide repeat loci. The polymerase has been engineered to lack both 5′ and 3′ exonuclease activities and is severely restricted in its ability to add a nontemplated nucleotide to the end of the PCR product. It can be substituted directly for *Taq* DNA polymerase in amplification reactions as a simple solution to the heterogenous extra nucleotide addition problem. It is recommended for amplification of fragments up to 500 bp in length. The enzyme is supplied complexed with proprietary antibody that inhibits polymerase activity. Due to specific binding of the inhibitor, Platinum® GenoTYPE *Tsp* DNA Polymerase is provided in an inactive form. This reagent provides an automatic "hot start" for use in PCR (1,2). Hot starts are typically used in PCR to increase sensitivity, specificity and yield while allowing assembly of reactions at ambient temperatures. The extra time, effort, and contamination risks associated with manual hot start procedures are addressed with the use of Platinum® GenoTYPE *Tsp* DNA Polymerase. The activity of Platinum® GenoTYPE *Tsp* DNA Polymerase is blocked at ambient temperatures but is regained after the denaturation step in PCR cycling at 94°C.

Components

Platinum® GenoTYPE *Tsp* DNA Polymerase	11448-032
10X PCR Buffer, Minus Mg	Y02028
50 mM Magnesium Chloride	Y02016

Storage Buffer: 20 mM Tris-HCl (pH 8.0), 40 mM NaCl, 2 mM Sodium Phosphate, 0.1 mM EDTA, 1 mM DTT, stabilizers, 50% (v/v) glycerol

10X PCR Buffer: 200 mM Tris-HCl (pH 8.4), 500 mM KCl

For technical support, email tech_support@invitrogen.com.
For country-specific contact information, visit **www.invitrogen.com**.

Unit Definition

One *Tsp* unit of Platinum® GenoTYPE *Tsp* DNA Polymerase has been functionally determined to be equivalent to one unit of *Taq* DNA Polymerase in amplification of dinucleotide repeats using standard *Taq* reaction conditions. One *Tsp* unit approximates 2.5 activity units. An activity unit incorporates 10 nmol of deoxyribonucleotide into acid-precipitable material in 30 min at 74°C under optimized reaction conditions.

Quality Control

The Certificate of Analysis (CofA) provides detailed quality control information for each product. The CofA is available on our website at www.invitrogen.com/cofa, and is searchable by product lot number, which is printed on each box.

Protocol

The following general procedure is suggested as a guideline and as a starting point when using Platinum® GenoTYPE *Tsp* DNA Polymerase in any PCR amplification.

1. Add the following components to the PCR reaction tube:

Components	Volume	Final Concentration
10X PCR Buffer, Minus Mg	1.5 µl	1X
10 mM dNTP mixture	0.3 µl	0.2 mM each
50 mM $MgCl_2$	0.45 µl	1.5 mM
Primer mix (5 µM each)	1 µl	0.33 µM each
Template DNA	as required	50 ng
Platinum® GenoTYPE *Tsp* DNA Polymerase	0.12 µl	0.6 units
Autoclaved, distilled water	to 15 µl	Not applicable

If desired, a master mix can be prepared for multiple reactions, to minimize reagent loss and to enable accurate pipetting.

2. Perform 30 cycles of PCR amplification as follows:

Predenaturation	94°C for 1-2 min (if desired)
Denature	94°C for 30 s
Anneal	55°C for 30 s
Extend	72°C for 1 min for 10 cycles
Denature	89°C for 30 s
Anneal	55°C for 30 s
Extend	72°C for 1 min for 20 cycles
Final extension	72°C for 10 min (if desired)

3. Maintain the reaction at 4°C after cycling. The samples can be stored at -20°C until use.
4. Analyze the amplification products by electrophoresis. Use appropriate molecular weight standards to determine the size of the products.

References

1. Chou, Q., *et al*. (1992) *Nucl. Acids Res.*, *20*, 1717.
2. Sharkey, D.J., *et al*. (1994) *BioTechnology*, *12*, 506.

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