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Platinum® *Taq* Antibody

Cat. nos.	Size	Conc. 5 U/µL
10965-010	100 units	Store at –30°C to –10°C

Pub. Part no. 10965010.pps MAN0000922 **Rev. Date** 19 Dec 2011

Description

Platinum® *Taq* Antibody is a proprietary inhibitor which, when bound to *Taq* DNA polymerase, inactivates polymerase activity. This reagent provides an automatic "hot start" for *Taq* DNA polymerase in PCR.[1,2,3] 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® *Taq* Antibody. Platinum® *Taq* Antibody blocks *Taq* DNA polymerase activity at ambient temperatures but releases active polymerase after the denaturation step in PCR cycling at 94°C. By increasing the effectiveness of *Taq* DNA polymerase through use of this product, it is possible to reduce the optimization and handling of reaction components and improve PCR results. Platinum® *Taq* Antibody is supplied in a concentration sufficient to eliminate non-specific priming during amplification. To prepare an antibody:polymerase complex, Platinum® *Taq* Antibody is mixed with an equal number of units of *Taq* DNA polymerase. The mixture is used at the diluted enzyme unit concentration with no other modifications to PCR reactions necessary. Other native, recombinant, or truncated forms of *Taq* DNA polymerase,[4,5] including Elongase® Enzyme (Catalog Number 10480-010/-028), are also effectively inhibited.

Product Use: For research use only.
Not intended for any animal or human therapeutic or diagnostic use.

Unit Definition

One unit of Platinum® *Taq* Antibody is the amount of product required to inhibit one unit of *Taq* DNA polymerase.

One unit of *Taq* DNA polymerase incorporates 10 nmol of deoxyribonucleotide into DNA in 30 minutes at 74°C.

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.

PCR Precautions

PCR is a powerful technique capable of amplifying trace amounts of DNA, and precautions are required to avoid contamination. Amplification reactions should be assembled in a DNA-free environment. Use of aerosol-resistant barrier tips is recommended. Avoid cross-contamination with the primers or template DNA used in individual reactions. PCR products should be analyzed in an area separate from the reaction assembly area.

Protocol

The following general procedure is suggested as a starting point when using Platinum® *Taq* Antibody for a 50 µL PCR reaction. Reaction size may be altered to suit user preferences. Optimal reaction conditions (incubation times and temperatures, concentration of *Taq* DNA polymerase, primers, $MgCl_2$, and template DNA) may need to be optimized.

1. Measure out the amount of *Taq* DNA polymerase needed. When using a reaction cocktail, determine the total number of units in the mix.
2. Add a number of "equivalent units" of Platinum® *Taq* Antibody equal to the amount of *Taq* DNA polymerase units in the cocktail. For *Taq* DNA polymerase at a concentration of 5 U/µL, prepare a 1:1 mixture. Mix well using a Vortex Mixer.

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3. Add the following components to a sterile 0.5-mL microcentrifuge tube:

Components	Volume	Final Concentration
10X PCR Buffer, Minus Mg	5 µL	1X
10 mM dNTP mixture	1 µL	0.2 mM each
50 mM $MgCl_2$	1.5 µL	1.5 mM
Primer mix (10 µM each)	1 µL	0.2 µM each
Template DNA	≥1 µL	(as required)
Platinum® *Taq* Antibody: *Taq* DNA polymerase	1 µL	2.5 units (or as required)
Autoclaved, distilled water	to 50 µL	Not applicable

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

4. Mix the contents of the tubes cap the tubes and centrifuge briefly to collect the contents. Depending on the thermal cycler used, overlay with mineral or silicone oil if necessary.

5. Incubate tubes in a thermal cycler at 94°C for 30 seconds to 2 minutes to completely denature the template and activate the enzyme.

6. Perform 25–35 cycles of PCR amplification as follows:

Denature	94°C for 30 seconds
Anneal	55°C for 30 seconds
Extend	72°C for 1 minutes per kb

7. Maintain the reaction at 4°C after cycling. The samples can be stored at -20°C until use.

8. Analyze the amplification products by agarose gel electrophoresis. We recommend using E-Gel® 1.2% gels and TrackIt™ 100 bp or 1kb Plus DNA ladders (see **Additional Products** on page 4).

References

1. Chou, Q., *et al*. (1992) *Nucl. Acids Res., 20*, 1717.
2. Sharkey, D.J., *et al*. (1994) *BioTechnology, 12*, 506.
3. Westfall, B.A., *et al*. (1997) *Focus®, 19.3*, 46.
4. Barnes, W.M. (1992) *Gene, 112*, 29.
5. Lawyer, F.C., *et al*. (1993) *PCR Methods and Applications, 2*, 275.

Additional Products

Product	Amount	Catalog no.
E-Gel® 1.2% Starter Pak	6 gels plus PowerBase™	G6000-01
E-Gel® 1.2% 18-Pak	18 gels	G5018-01
TrackIt™ 100 bp DNA Ladder	100 applications	10488-058
TrackIt™ 1kb Plus DNA Ladder	100 applications	10488-085

Product Qualification and SDS

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to **www.lifetechnologies.com/support** and search for the Certificate of Analysis by product lot number, which is printed on the box.

Safety Data Sheets (SDSs) are available at **www.lifetechnologies.com/sds**.

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