Certificate of Analysis

Titanium® DNA Amplification Kit

Catalog No.	Amount	Lot Number
639243	400 rxns	2412166A

Description

Titanium *Taq* is a mixture consisting of a 5′ exonuclease-deficient *Taq* polymerase and TaqStart® Antibody, a monoclonal antibody which inhibits Titanium *Taq* at ambient temperatures. TaqStart Antibody provides automatic hot start PCR. An optimized buffer mix (yielding a final concentration of 3.5 mM $MgCl_2$) and a purified mixture of dNTPs (2.5 mM each) is also included. The RNase-free GC-Melt Reagent (5M) improves the specificity and yield of PCR reactions, especially when using templates with a high GC-content or complex secondary structure. This kit contains sufficient reagents for Titanium reactions of 100 µl each. The Titanium DNA Amplification Kit is designed to be used with Affymetrix DNA Mapping products (see Table 1).

Package Contents

- 0.9 ml 50X Titanium *Taq* DNA Polymerase Mix
- 4.5 ml 10X Titanium Taq PCR Buffer
- 6.2 ml dNTP Mixture (2.5 mM each)
- 8.9 ml GC-Melt Reagent (5.0 M)

Storage Conditions

- Store at –20°C.

NOTE: At times, precipitate may be observed in the GC-Melt. This precipitate does not affect the performance of the kit. The precipitate can be dissolved rapidly by mixing at room temperature or warming at 37°C for a few minutes.

Expiration Date

- JAN. 21, 2028

Shipping Conditions

- Dry ice

Quality Control Data

Raw Material Quality Control

Purified N-terminal deletion mutant *Taq* polymerase was tested for enzymatic activity and PCR performance. Endonuclease, exonuclease, and DNA contamination assays were also performed.

PCR Performance

N-terminal deletion mutant *Taq* polymerase was serially diluted (0.6–0.2 µg/µl) and each serial dilution used in a separate PCR reaction with λ genomic DNA as a template. The optimal protein concentration per reaction was determined as the amount of protein (in µg) required to amplify >20 ng/µl of a 3.5 kb λ fragment with minimal background.

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(070524)

Functional Quality Control

Amplification from a cDNA template
Titanium *Taq* DNA polymerase and 10X Titanium Buffer were tested in a 50 µl PCR reaction using 5 µl of Marathon®-Ready Human Placenta cDNA (Cat. No. 639311) and control primers specific for a 1.3 kb fragment from the transferrin receptor (TFRC) gene (0.2 µM each). Conditions were set at:

95°C	1 min
30 cycles:	
98°C	15 sec
68°C	1.5 min

5 µl of the PCR product was electrophoresed on a 1.2% TAE/agarose gel to confirm the presence of a 1.3 kb band with minimal background. PCR product concentration was measured by fluorometry. The yield was determined to be ≥5 ng/µl.

Amplification from a genomic DNA template
Titanium *Taq* DNA polymerase and 10X Titanium Buffer were tested in a 50 µl PCR reaction using 100 ng of calf thymus genomic DNA as a template and control primers specific for a 407 bp fragment of the bovine pancreatic trypsin inhibitor (BPTI) gene (0.4 µM each). Conditions were set at:

94°C	3 min
30 cycles:	
94°C	30 sec
68°C	1.5 min

5 µl of PCR product was electrophoresed on a 1.2% TAE/agarose gel to confirm the presence of a 407 bp band with minimal background. PCR product concentration was measured by fluorometry. The yield was determined to be ≥15 ng/µl.

Amplification from a GC-rich template
The GC-Melt Reagent (5M) was tested using the reagents from the Advantage® GC 2 PCR Kit (Cat. Nos. 639120 & 639119). Amplification of a 510 bp GC-rich fragment from the insulin-like growth factor receptor II (IGFR II) gene, of which 110 bp are 90% GC-rich, was performed using the kit reagents in the presence of increasing concentrations of GC-Melt Reagent (0, 0.5, 1.0, 1.5, and 2 M). Reactions were assembled and performed as described in the Advantage GC 2 User Manual, PT3316-1. Cycle parameters were set as follows:

94°C	3 min
25 cycles:	
94°C	30 sec
68°C	1.5 min
68°C	3 min

5 µl of each PCR product was electrophoresed on a 2.0% agarose/EtBr gel. The presence of a major band of 0.5 kb was observed in the lanes containing template amplified in the presence of 0.5 M and 1.0 M GC-Melt Reagent.

dNTP Mixture (2.5 mM each)
Individual dNTP were tested for purity by HPLC assay before mixing. Each is more than 98% pure.

dNTP materials	HPLC purity
dATP	99.8%
dCTP	99.9%
dGTP	99.9%
dTTP	99.9%

The dNTP Mixture (2.5 mM each) was tested in a PCR reaction using λ DNA as a template. Amplification of both 500 bp and 20 kb fragments was observed. In addition, the dNTP mixture was tested in an RT-PCR reaction using total RNA from HL60 cells as a template. Amplification of a 4.4 kb fragment was observed using a human TFRC gene cDNA template.

Table 1. Affymetrix and Takara Bio Kit Cat. Nos.

	Affymetrix Cat. Nos.			Takara Bio Cat. Nos.	
Product	**30 Rxn Kits**	**100 Rxn Kits**	**User Manual**	**639240 300 Rxn Kit**	**639243 400 Rxn Kit**
GeneChip Mapping 250K Nsp Assay Kit	900766	900753	701930	✓	—
GeneChip Mapping 250K Sty Assay Kit	900765	900754	701930	✓	—
Affymetrix Genome-Wide Human SNP Nsp/Sty Assay Kit 5.0	901013	901015	702419	✓	✓

It is certified that this product meets the above specifications, as reviewed and approved by the Quality Department.

Notice to Purchaser

Titanium® DNA Amplification Kit

CATALOG NO.

639243

NOTICE TO PURCHASER:

Our products are to be used for **Research Use Only**. They may not be used for any other purpose, including, but not limited to, use in humans, therapeutic or diagnostic use, or commercial use of any kind. Our products may not be transferred to third parties, resold, modified for resale, or used to manufacture commercial products or to provide a service to third parties without our prior written approval.

Your use of this product is also subject to compliance with the licensing requirements, listed below if applicable, and described on the product´s web page at http://www.takarabio.com. It is your responsibility to review, understand and adhere to any restrictions imposed by these statements.

TRADEMARKS:

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