

Certificate of Analysis

D16S539 Add-In for PowerPlex® 1.1:

Cat.#
DK3131

Description: D16S539 Add-In for PowerPlex® 1.1^(a) is provided to alleviate a potential allele dropout in the D16S539 locus of the PowerPlex® 1.1 System (Cat.# DC6090 and DC6091) when amplified with AmpliTaq Gold® and Gold ST ★R 10X Buffer. Laboratories using AmpliTaq Gold® and Gold ST ★R 10X Buffer have reported allele dropout in approximately 1.2% of samples in the D16S539 locus. This allele dropout has been confirmed at Promega Corporation. This mutation is not detected, and allele dropout does not occur when Taq DNA Polymerase and STR 10X Buffer are used with the system.

To prevent the allele dropout, a second primer pair containing the sequence mutation can be added to the amplification master mix. The D16S539 Add-In for PowerPlex® 1.1 is provided as a 10X primer pair. Also included is the Gold ST ★R 10X Buffer required for use with the AmpliTaq Gold® DNA polymerase.

Components: D16S539 Add-In for PowerPlex® 1.1 (250µl) and Gold ST ★R 10X Buffer (300µl).

Storage Conditions: Store at -20°C.

Quality Control Assays

Performance Testing: One human genomic DNA that is heterozygous for the D16S539 locus (shows allele dropout), titrated to 1.0 and 0.5ng, and one negative control (without DNA) are amplified individually using PowerPlex® 1.1 10X Primer Pair Mix, AmpliTaq Gold®, Gold ST ★R 10X Buffer and D16S539 Add-In for PowerPlex® 1.1. The same set of DNA samples are amplified using PowerPlex® 1.1 10X Primer Pair Mix, AmpliTaq Gold® and Gold ST ★R 10X Buffer without D16S539 Add-In for PowerPlex® 1.1. All the amplified samples, mixed with Bromophenol Blue Loading Solution, are loaded on a denaturing polyacrylamide gel. After electrophoretic separation, the gel is analyzed using a Hitachi FMBIO® II Fluorescent Scanner.

All protocols are provided in the **Usage Information (reverse side)** and the *PowerPlex® 1.1 System Technical Manual* (#TMD008).

Minimum Specifications:

- All alleles must be detectable at 0.5ng DNA.
- The correct allele sizes in repeat numbers from the amplified DNA samples tested with D16S539 Add-In for PowerPlex® 1.1 are 11,10 for the D16S539 locus.
- The correct allele sizes in repeat numbers from the amplified DNA samples tested without D16S539 Add-In for PowerPlex® 1.1 are 11,11 for the D16S539 locus.
- The negative DNA control reaction must be devoid of amplification products.

^(a)STR loci are the subject of U.S. Pat. No. RE 37,984, German Pat. No. DE 38 34 636 C2 and other patents issued to the Max-Planck-Gesellschaft zur Förderung der Wissenschaften, e.V., Germany. The development and use of STR loci are covered by U.S. Pat. No. 5,364,759, Australian Pat. No. 670231 and other pending patents assigned to Baylor College of Medicine, Houston, Texas.

Use of Promega's STR Systems requires performance of the polymerase chain reaction (PCR), which is the subject of European Pat. Nos. 201,184 and 200,362 owned by Hoffmann-La Roche*. Purchase of Promega's STR Systems does not include or provide a license with respect to these patents or any other PCR-related patent owned by Hoffmann-La Roche or others. Users of Promega's STR Systems may, therefore, be required to obtain a patent license, depending on the country in which the systems are used. For more specific information on obtaining a PCR license, please contact Hoffmann-La Roche.

*The above primary European Pat. Nos. 201,184 and 200,362 will expire on March 28, 2006. In the U.S., the patents covering the foundational PCR process expired on March 29, 2005.

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I. Amplification Protocol

Follow the protocols in the *PowerPlex® 1.1 System Technical Manual* (#TMD008, Section V.B) with the following exceptions:

A. Amplification Setup

1. Use Gold ST ★R 10X Buffer when using AmpliTaq Gold®. The STR 10X Buffer (pH 9.0), supplied with the PowerPlex® 1.1 System, is not compatible with AmpliTaq Gold® because the optimal pH for the modified *Taq* DNA Polymerase is 8.3.
2. The Gold ST ★R 10X Buffer contains BSA. No addition of BSA is required.
3. Ice is not needed when setting up reactions using AmpliTaq Gold®.
4. Use the following versions of Tables 9 and 10 (described in the *PowerPlex® 1.1 System Technical Manual* #TMD008) for the Master Mix.

Table 9. Master Mix for the PowerPlex® 1.1 System.

PCR Master Mix Component	Volume Per Sample	Number of Reactions	Final Volume (µl)
Nuclease-Free Water	14.6µl		
Gold ST ★R 10X Buffer	2.5µl		
PowerPlex® 1.1 10X Primer Pair Mix	2.5µl		
D16S539 Add-In for PowerPlex® 1.1	2.5µl		
AmpliTaq Gold® DNA polymerase*	0.4µl (2u)		
Total Volume	22.5µl		

Table 10. Master Mix for the PowerPlex® 1.1 System and Amelogenin (TMR).

PCR Master Mix Component	Volume Per Sample	Number of Reactions	Final Volume (µl)
Nuclease-Free Water	12.05µl		
Gold ST ★R 10X Buffer	2.5µl		
PowerPlex® 1.1 10X Primer Pair Mix	2.5µl		
Amelogenin 10X Primer Pair (TMR)	2.5µl		
D16S539 Add-In for PowerPlex® 1.1	2.5µl		
AmpliTaq Gold® DNA polymerase*	0.45µl (2.25u)		
Total Volume	22.5µl		

*Tables 9 and 10 assume that the AmpliTaq Gold® DNA polymerase is at a concentration of 5u/µl. If the enzyme concentration is different, the volume of enzyme used must be adjusted accordingly.

B. Amplification Thermal Cycling

When using AmpliTaq Gold®, an additional incubation at 95°C for 11 minutes must be incorporated prior to initiation of the thermal cycling program.