TECHNICAL MANUAL

PunchSolution™ Kit

Instructions for Use of Product
DC9271

Revised 9/16
TMD038

PunchSolution™ Kit

All technical literature is available at: www.promega.com/protocols/
Visit the web site to verify that you are using the most current version of this Technical Manual.
E-mail Promega Technical Services if you have questions on use of this system: genetic@promega.com

1. Description

The PunchSolution™ Kit is designed for processing punches from nonFTA storage cards prior to amplification using PowerPlex® Systems for human STR genotyping. The PunchSolution™ Kit is suitable for preprocessing punches from nonFTA storage cards that have been used to collect buccal cells or blood. The PunchSolution™ Kit contains PunchSolution™ Reagent and 5X AmpSolution™ Reagent. The PunchSolution™ Reagent is used for treating punches before amplification. The 5X AmpSolution™ Reagent must be added to amplification reactions when performing direct amplification of DNA from treated punches under the conditions indicated in Table 1. It is not possible to generate a full STR profile under the conditions stated in Table 1 without the addition of 5X AmpSolution™ Reagent.

1. Description (continued)

Table 1. Amplification Conditions Requiring 5X AmpSolution™ Reagent.

PowerPlex® System	Direct-Amplification Reaction Volume
PowerPlex® Fusion 6C[1]	12.5µl
PowerPlex® Fusion[1]	12.5µl
PowerPlex® Y23[1]	12.5µl
PowerPlex® 21[1]	12.5µl
PowerPlex® 18D[1]	12.5µl
PowerPlex® ESX Fast and ESI Fast	12.5µl and 25µl
PowerPlex® ESX and ESI	25µl
PowerPlex® CS7	25µl
PowerPlex® 16 HS	12.5µl and 25µl
PowerPlex® 16[2]	12.5µl and 25µl

[1]5X AmpSolution™ Reagent is not required with these PowerPlex® Systems in a 25µl direct-amplification reaction volume.
[2]The PunchSolution™ Kit and PowerPlex® 16 System should only be used with buccal samples, not blood samples.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
PunchSolution™ Kit	**100 preparations**	**DC9271**

Not For Medical Diagnostic Use. This system contains sufficient reagents to process 100 samples. Includes:

- 1ml PunchSolution™ Reagent
- 500µl 5X AmpSolution™ Reagent

Storage Conditions: Upon arrival, thaw the PunchSolution™ Reagent completely, mix by gentle inversion and store at 2–10°C. Thaw the 5X AmpSolution™ Reagent completely (in a 37°C water bath or at ambient temperature), mix by vortexing and store at 2–10°C. The 5X AmpSolution™ Reagent may be turbid after thawing or storage at 4°C. If this occurs, warm the reagent briefly at 37°C, then vortex until clear. Do not store reagents in the refrigerator door, where the temperature can fluctuate. Storing reagents in the refrigerator door can compromise stability.

The 5X AmpSolution™ Reagent is used with certain direct-amplification reaction conditions of PowerPlex® Systems (see Table 1). This reagent is not required for the PowerPlex® Fusion 6C, PowerPlex® Fusion, PowerPlex® Y23, PowerPlex® 21 and PowerPlex® 18D Systems when using a 25µl amplification reaction volume. The reagent is required for the PowerPlex® Fusion 6C, PowerPlex® Fusion, PowerPlex® Y23, PowerPlex® 21, PowerPlex® 18D, PowerPlex® ESX Fast and ESI Fast and PowerPlex® 16 HS Systems when amplifying DNA directly from storage card punches in a 12.5µl reaction volume. We do not recommend using the PowerPlex® 16 System for punches from blood cards. Inclusion of the 5X AmpSolution™ Reagent in the PowerPlex® 16 System reactions with blood punches may not overcome the inhibition caused by heme carryover.

3. Protocol for Processing 1.2mm Punches from NonFTA Storage Cards

NonFTA sample types include:

- buccal samples on Bode Buccal DNA Collector™ devices
- blood or buccal samples on nonFTA storage card punches (e.g., S&S 903)

Materials to Be Supplied by the User

- MicroAmp® optical 96-well reaction plate (Applied Biosystems Part# N801-0560) or equivalent
- 70°C heat block with insert capable of accepting a 96-well plate or thermal cycler set to 70°C

1. Place one 1.2mm punch per sample into a well of the 96-well reaction plate.

   Note: In this protocol, the punch is added to the empty well, and then the PunchSolution™ Reagent is added. Adding PunchSolution™ Reagent to the well before adding the punch also is acceptable and may help alleviate static problems.

2. Add 10µl of PunchSolution™ Reagent to each 1.2mm punch.

   Note: Do not cover the plate with a lid or sealer or place the plate in a thermal cycler with a closed, heated lid. A closed, heated lid will prevent evaporation, even with an open plate. If you use a thermal cycler with a heated lid, leave the lid open to allow efficient evaporation.

3. Incubate plate at 70°C for 30 minutes or until wells are dry.

   Note: We strongly recommend incubating for the full 30 minutes. Shorter incubation times may result in poor performance.

We highly recommend the use of gloves and aerosol-resistant pipette tips.

4. Amplification and Detection of Amplified Fragments

Follow the amplification protocol for Direct Amplification of DNA from Storage Card Punches and the standard detection protocol in the appropriate PowerPlex® System Technical Manual to amplify DNA from storage card punches and prepare samples for injection on the capillary electrophoresis (CE) instrument.

5. Troubleshooting

The following troubleshooting section is specific for the PunchSolution™ Kit. For additional troubleshooting information pertaining to the specific PowerPlex® System you are using, please refer to the troubleshooting section of that PowerPlex® System Technical Manual, which is available online at: **www.promega.com/protocols/**

For questions not addressed here or in the troubleshooting section of the PowerPlex® System Technical Manual, please contact your local Promega Branch Office or Distributor. Contact information available at: **www.promega.com**. E-mail: **genetic@promega.com**

Symptoms	Causes and Comments
Faint or absent allele peaks	Poor sample deposition. Shedding and collection of donor cells was variable. Increase cycle number.
	Active PunchSolution™ Reagent carried over into the PowerPlex® System reaction. Ensure that the heat block reached 70°C and samples were incubated for 30 minutes or until wells are dry. Incubation for shorter time periods may result in incomplete inactivation of the PunchSolution™ Reagent. We have not tested longer incubation times.
	Inactive PunchSolution™ Reagent. Thaw PunchSolution™ Reagent completely and mix by gentle inversion. Store thawed PunchSolution™ Reagent at 2–10°C in a refrigerator. Do not store reagents in the refrigerator door, where the temperature can fluctuate. Do not refreeze, as this may reduce activity.
	If using amplification conditions that require 5X AmpSolution™ Reagent (see Table 1), make surethat the PCR amplification mix contained AmpSolution™ Reagent. Omitting AmpSolution™ Reagent from these PowerPlex® System reactions will result in amplification failure.

Symptoms	Causes and Comments
Faint or absent peaks seen for positive control DNA	If the positive control DNA supplied with the PowerPlex® System fails to amplify, make sure that the correct amount of positive control DNA was added to the amplification reaction. Due to the reduced cycle numbers used with processed punches it is necessary to increase the mass of positive control DNA to obtain a profile. Follow the recommendation provided in the appropriate PowerPlex® System Technical Manual.
Extra peaks visible in one or all color channels	Processed 1.2mm punch was contaminated.
	Amplification of processed punches with high DNA concentrations can result in artifact peaks due to overamplification, resulting in saturating signal on the CE. Using more than the recommended number of storage card punches per reaction may result in overamplification and signal saturation. If the signal is saturated, repeat the amplification with a smaller punch, a larger reaction volume or reduced cycle number to reduce peak heights.
	Amplification of excess template for a given cycle number can result in overloading of the capillary upon electrokinetic injection. Excess DNA in the capillary is difficult to maintain in a denatured single-stranded state. Some single-stranded DNA renatures and becomes double-stranded. Double-stranded DNA migrates faster than single-stranded DNA during capillary electrophoresis and appears as "shadow" peaks migrating in front of the main peaks. If this occurs at a heterozygous locus, it is sometimes possible to see two "shadow" peaks that differ in size by approximately the same distance as the single-stranded alleles. Repeat the amplification with fewer punches or reduced cycle number.

5. Troubleshooting (continued)

Symptoms	Causes and Comments
Peak height imbalance	Excess DNA in PowerPlex® System reactions can result in locus-to-locus imbalance within a dye channel such that the peak heights at the smaller loci are greater than those at the larger loci (ski-slope effect). Use a smaller punch, a larger reaction volume or reduced cycle number.
	Active PunchSolution™ Reagent carried over into PowerPlex® System reaction. Larger loci are most susceptible to active PunchSolution™ Reagent carryover and will drop out before the smaller loci. Ensure that the heat block reached 70°C and samples were incubated for 30 minutes or until wells are dry. Incubation for shorter time periods may result in incomplete inactivation of the PunchSolution™ Reagent.
	Inactive PunchSolution™ Reagent. Thaw PunchSolution™ Reagent completely and mix by gentle inversion. Store thawed PunchSolution™ Reagent at 2–10°C in a refrigerator. Do not store reagents in the refrigerator door, where the temperature can fluctuate. Do not refreeze, as this may reduce activity.
	Carryover of active PunchSolution™ Reagent into amplification reaction. We recommend treating one 1.2mm punch with 10µl of PunchSolution™ Reagent per recommended amplification reaction. Use of a smaller amplification reaction volume may compromise performance if using 10µl of PunchSolution™ Reagent. Reduction of PunchSolution™ Reagent volume may improve results for reduced amplification reaction volume. Laboratory optimization and validation are required.
Extreme variability in sample to sample peak heights	There can be significant individual-to-individual variability in the deposition of cells onto a punch, resulting in peak height variability between samples. The PunchSolution™ Kit increases the recovery of DNA from samples but does not normalize the amount of DNA present.

6. Related Products

Product	Size	Cat.#
SwabSolution™ Kit	100 preps	DC8271
5X AmpSolution™ Reagent	500µl	DM1231
PowerPlex® Fusion 6C System	50 reactions	DC2705
	200 reactions	DC2720
PowerPlex® Fusion System	200 reactions	DC2402
	800 reactions	DC2408
PowerPlex® ESX 17 Fast System	100 reactions	DC1711
	400 reactions	DC1710
PowerPlex® ESI 17 Fast System	100 reactions	DC1721
	400 reactions	DC1720
PowerPlex® ESX 16 Fast System	100 reactions	DC1611
	400 reactions	DC1610
PowerPlex® ESI 16 Fast System	100 reactions	DC1621
	400 reactions	DC1620
PowerPlex® 18D System	200 reactions	DC1802
	800 reactions	DC1808
PowerPlex® 21 System	200 reactions	DC8902
	4 × 200 reactions	DC8942
PowerPlex® 16 HS System	100 reactions	DC2101
	400 reactions	DC2100
PowerPlex® 16 System	100 reactions	DC6531
	400 reactions	DC6530
PowerPlex® ESX 16 System	100 reactions	DC6711
	400 reactions	DC6710
PowerPlex® ESX 17 System	100 reactions	DC6721
	400 reactions	DC6720
PowerPlex® ESI 16 System	100 reactions	DC6771
	400 reactions	DC6770
PowerPlex® ESI 17 Pro System	100 reactions	DC7781
	400 reactions	DC7780
PowerPlex® CS7 System	100 reactions	DC6613

6. Related Products (continued)

Product	Size	Cat.#
PowerPlex® Y23 System	50 reactions	DC2305
	200 reactions	DC2320

Not for Medical Diagnostic Use.

7. Summary of Changes

The following change was made to the 9/16 revision of this document:

1. A note was added to Section 3 for clarity.