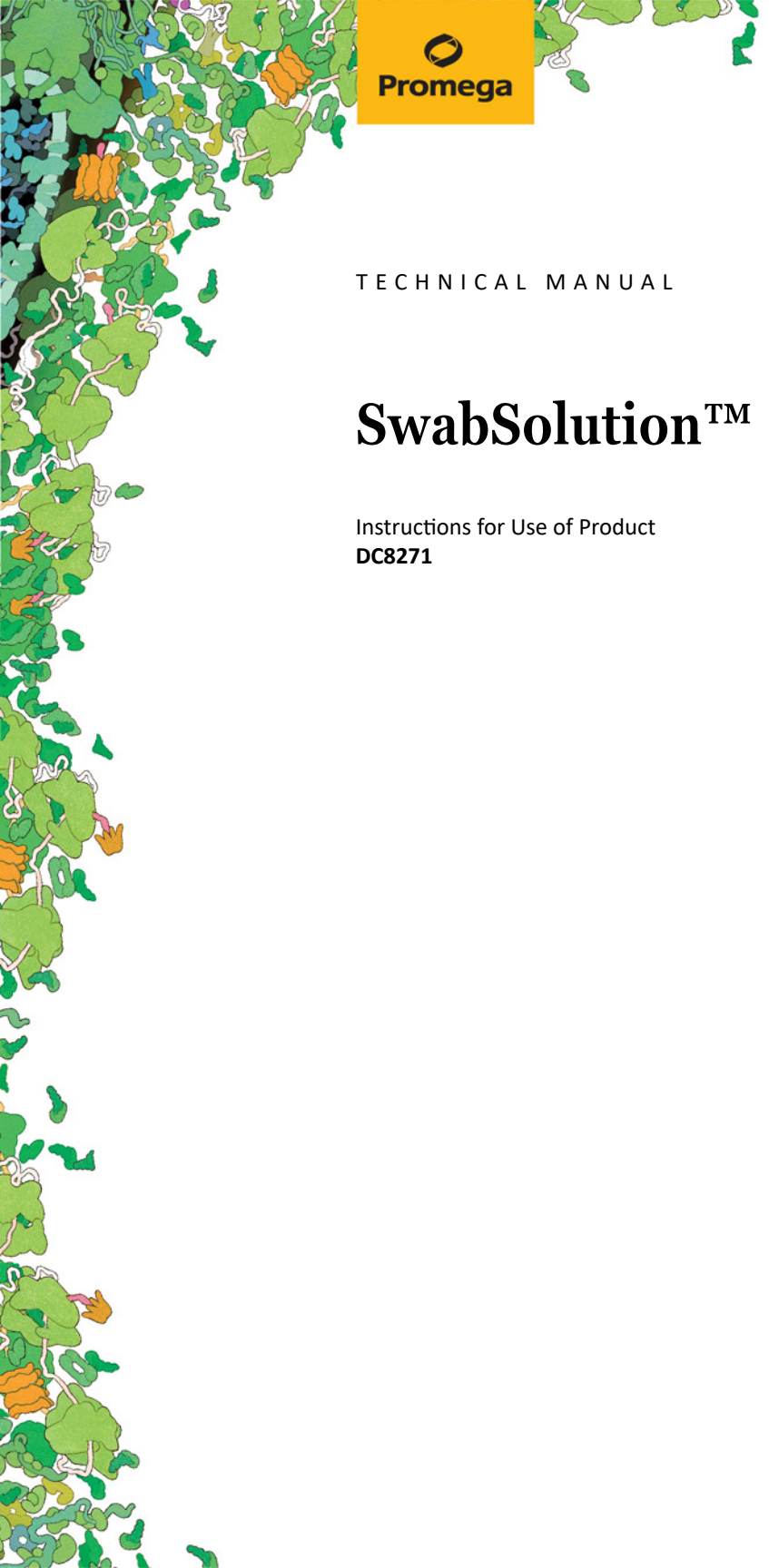

TECHNICAL MANUAL

SwabSolution™ Kit

Instructions for Use of Product
DC8271

Revised 4/21
TMD037

Promega

SwabSolution™ 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

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1. Description

The SwabSolution™ Kit is used for rapid processing of swabs prior to amplification using PowerPlex® Systems for human STR genotyping. The SwabSolution™ Kit contains SwabSolution™ Reagent, which is used to generate a buccal swab extract that is added to the PowerPlex® System reaction. In addition, the SwabSolution™ Kit contains 5X AmpSolution™ Reagent, which must be added to amplifications with certain PowerPlex® Systems when performing direct amplification of DNA from swabs (see Table 1). These PowerPlex® Systems will not generate a full STR profile from swab extracts without the addition of this 5X AmpSolution™ Reagent.

Table 1. PowerPlex® System Requirement for 5X AmpSolution™ Reagent.

PowerPlex® Systems Requiring 5X AmpSolution™ Reagent	PowerPlex® Systems NOT Requiring 5X AmpSolution™ Reagent
PowerPlex® ESX Fast and ESI Fast	PowerPlex® Fusion 6C
PowerPlex® ESX and ESI	PowerPlex® Fusion
PowerPlex® CS7	PowerPlex® Y23
PowerPlex® 16 HS	PowerPlex® 21
PowerPlex® 16	PowerPlex® 18D

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
SwabSolution™ Kit	**100 preparations**	**DC8271**

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

- 100ml SwabSolution™ Reagent
- 500µl 5X AmpSolution™ Reagent

Storage Conditions: Upon arrival, thaw the SwabSolution™ Reagent completely in a 37°C water bath and mix by gentle inversion. After thawing, store at +2°C to +10°C. Thaw the 5X AmpSolution™ Reagent completely (in a 37°C water bath or at ambient temperature) and mix by vortexing before storing at +2°C to +10°C. The 5X AmpSolution™ Reagent may be turbid after thawing or storage at +2°C to +10°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 for amplification of swab extracts with certain PowerPlex® Systems (see Table 1). This reagent is not required for the PowerPlex® Fusion 6C, PowerPlex® Fusion, PowerPlex® 18D, PowerPlex® 21 and PowerPlex® Y23 Systems.

3. Protocol for Processing Buccal Swabs

Materials to Be Supplied by the User

- ClickFit Microtube, 1.5ml (Cat.# V4745), or other 1.5ml microcentrifuge tube with locking cap or screw top or 2.2ml, Square-Well Deep Well Plate (Cat.# V6781)
- heat block set to 70°C for 1.5ml tubes or 90°C for 2.2ml Square-Well Deep Well Plates
- Heat Block Adapter (Cat.# A2661) if processing buccal swabs in a 2.2ml Square-Well Deep Well Plate; the Heat Block Adapter sits on top of the heat block and allows efficient heat transfer to the plate

When using the tube format, make sure to use 1.5ml microcentrifuge tubes with locking caps or screw tops, such as ClickFit Microtube, 1.5ml (Cat.# V4745), to ensure that the caps will not open during the 70°C incubation.

When using the 2.2ml Square-Well Deep Well Plate format, set the heat block to 90°C to ensure that the contents of each well reach 70°C.

Do not use an incubator to incubate tubes or plates. Heat transfer is inefficient and will result in poor performance. Only use a heat block to maintain efficient heat transfer.

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

3.A. Processing Buccal Swabs in Tubes

1. Set a heat block capable of accepting 1.5ml tubes to 70°C. The heat block must reach 70°C prior to the incubation in Step 4.
2. Place buccal swab head in a 1.5ml tube.
3. Add 1ml of SwabSolution™ Reagent to each buccal swab head. Close the tube.

   Note: You do not need to vortex after addition of SwabSolution™ Reagent prior to incubation or after the 30-minute incubation is complete.
4. Place tube in heat block, and incubate sample at 70°C for 30 minutes.

   Note: Buccal swab extracts may be stored at 4°C. In-house stability testing at Promega has shown that DNA can be amplified from extracts that have been stored for 4 years at 4°C.

3.B. Processing Buccal Swabs in a Deep-Well Plate

1. Place the Heat Block Adapter on a heat block that is set to 90°C. The heat block must reach 90°C prior to the incubation in Step 5.
2. Place each buccal swab head in an empty well of a 2.2ml Square-Well Deep Well Plate.
3. Add 1ml of SwabSolution™ Reagent to each buccal swab head.
4. Place the 2.2ml Square-Well Deep Well Plate on the preheated Heat Block Adapter. You do not need to seal the plate.

   Note: You do not need to vortex after addition of SwabSolution™ Reagent prior to incubation or after the 30-minute incubation is complete.
5. Incubate the 2.2ml Square-Well Deep Well Plate for 30 minutes.

   Note: Buccal swab extracts may be stored at 4°C. In-house stability testing at Promega has shown that DNA can be amplified from extracts that have been stored for 4 years at 4°C.

4. Amplification and Detection of Amplified Fragments

Follow the amplification protocol for Direct Amplification of DNA from Swabs and the standard detection protocol in the appropriate PowerPlex® System Technical Manual to amplify DNA from swab extracts and prepare samples for injection on the capillary electrophoresis (CE) instrument.

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5. Troubleshooting

The following troubleshooting section is specific for the SwabSolution™ 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.
	Inactive SwabSolution™ Reagent. Thaw SwabSolution™ Reagent completely in a 37°C water bath and mix by gentle inversion. Store thawed SwabSolution™ Reagent at +2°C to +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.
	Active SwabSolution™ Reagent carried over from swab extracts into the amplification reaction. Ensure that the heat block has reached 70°C (90°C if using a 2.2ml, Square-Well Deep Well Plate) and samples were incubated for the full 30 minutes. Incubation for shorter time periods may result in incomplete inactivation of SwabSolution™ Reagent. Do not use an incubator to incubate tubes or plates. Heat transfer is inefficient and will result in poor performance. Use only a heat block to maintain efficient heat transfer. We have tested 60-minute incubation times and observed no difference in performance compared to a 30-minute incubation.
	If using a PowerPlex® System that requires 5X AmpSolution™ Reagent (see Table 1), make sure that the PCR amplification mix contained AmpSolution™ Reagent. Omitting AmpSolution™ Reagent from these PowerPlex® System amplification reactions will result in amplification failure.
	If the positive control DNA supplied with the PowerPlex® System failed 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 swab extracts, it is necessary to increase the mass of positive control DNA. Follow the recommendation provided in the appropriate PowerPlex® System Technical Manual.

Symptoms	Causes and Comments
Extra peaks visible in one or all color channels	Swab extract was contaminated. Include a reagent blank as a negative control when processing samples.
	Artifacts of STR amplification. Amplification of swab extracts with high DNA concentrations can result in artifact peaks due to overamplification, resulting in saturating signal on the CE instrument. Using more than the recommended amount of swab extract as listed in the appropriate PowerPlex® System Technical Manual may result in overamplification and signal saturation. If signal is saturated, repeat amplification with less swab extract or reduced cycle number to reduce peak heights. Laboratory optimization and validation are required.
	Artifacts of STR amplification. Too much template DNA can result in artifacts that appear one base smaller than the allele. This is due to incomplete addition of the 3´ terminal A residue. Be sure to perform a final extension step at 60°C after thermal cycling as recommended in the PowerPlex® System Technical Manual. Repeat the amplification with less swab extract or reduced cycle number.
	Amplification of excess template for a given cycle number resulted 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 possible to see two “shadow” peaks that differ in size by approximately the same distance as the single-stranded alleles. Repeat the amplification with less swab extract or reduce cycle number.

5. Troubleshooting (continued)

Symptoms	Causes and Comments
Peak height imbalance	Excess DNA in the 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 less swab extract or reduce cycle number.
	Active SwabSolution™ Reagent carried over from swab extracts into the PowerPlex® System reaction. Larger loci are most susceptible to SwabSolution™ Reagent carryover and will drop out before the smaller loci. Ensure that the heat block has reached 70°C (90°C if using 2.2ml, Square-Well Deep Well Plates) and samples were incubated for the full 30 minutes. Incubation for shorter time periods may result in incomplete inactivation of SwabSolution™ Reagent. Do not use an incubator to incubate tubes or plates. Heat transfer is inefficient and will result in poor performance. Use only a heat block to maintain efficient heat transfer.
	Inactive SwabSolution™ Reagent. Thaw the SwabSolution™ Reagent completely in a 37°C water bath and mix by gentle inversion. Store thawed SwabSolution™ Reagent at +2°C to +10°C. Do not store reagents in the refrigerator door, where the temperature can fluctuate. Do not refreeze, as this may reduce activity.
Extreme variability in sample-to-sample peak heights	There can be significant individual-to-individual variability in the deposition of cells onto buccal swabs. This will appear as variability in peak heights between swab extracts. The extraction process increases the recovery of amplifiable DNA from buccal swabs but does not normalize the amount of DNA present. If variability is extreme, quantify the DNA from the swab extracts using a fluorescence-based double-stranded DNA (dsDNA) quantification method to determine the amount of total DNA or a qPCR-based quantification method for human DNA (see Section 6). The quantification values can be used to normalize input template amounts to minimize variation in signal intensity.

6. Appendix

The buccal swab extract generated with the SwabSolution™ Reagent may be added directly to a PowerPlex® System reaction. However, some laboratories may wish to quantify DNA to normalize the mass of input DNA. We have evaluated the compatibility of these extracts with human DNA-specific qPCR assays (e.g., Plexor® HY System) and fluorescence-based total DNA quantification assays (e.g., QuantiFluor® dsDNA System and Quant-iT™ PicoGreen® dsDNA Reagent).

6.A. Recommendations for PowerQuant® System

The amount of human genomic DNA present in buccal swab extracts can be quantified using the PowerQuant® System. Adding 5X AmpSolution™ Reagent to PowerQuant® amplification reactions is not required for quantification using the PowerQuant® System. Follow the protocol in the *PowerQuant® System Technical Manual* #TMD047 without any changes, using 2µl of buccal swab extract per 20µl PowerQuant® System amplification reaction. In addition, the human DNA quality in these extracts can be evaluated using the PowerQuant® data. The ratio of the autosomal-to-degradation target quantification results can indicate whether or not the human DNA is degraded, whereas the ratio of the autosomal-to-Y target quantification results can provide information on the ratio of female-to-male DNA in the sample. The performance of the PowerQuant® IPC target can indicate whether PCR inhibitors are present, which are likely to inhibit downstream STR amplification.

6.B. Recommendations for Plexor® HY System

The amount of human genomic DNA in buccal swab extracts may be quantified using the Plexor® HY System if 5X AmpSolution™ Reagent is added to the DNA quantification reaction. The SwabSolution™ Reagent will inhibit the qPCR amplification if 5X AmpSolution™ Reagent is not added to the reaction. Include the 5X AmpSolution™ Reagent in the DNA standard curve reactions as well as in the buccal swab extract amplifications. Modify the Plexor® HY reaction mix described in the reaction setup section of the *Plexor® HY System Technical Manual* as follows.

Component	Volume per Reaction
Water, Amplification Grade	3µl
Plexor® HY 2X Master Mix	10µl
Plexor® HY 20X Primer/IPC Mix	1µl
5X AmpSolution™ Reagent	4µl
Total volume	18µl

Add 2µl of extract to 18µl of Plexor® HY reaction mix to give the final 20µl reaction volume.

6.C. Recommendations for Fluorescent dsDNA Quantification Assay

Up to 10µl of a buccal swab extract generated with SwabSolution™ Reagent may be added to a 200µl fluorescent dsDNA quantification assay (typically 10µl of DNA sample in 90µl of TE buffer plus 100µl of a 1:200 dilution of the fluorescent dsDNA-binding dye reagent). The volume of swab extract should not exceed 5% of the total reaction volume (>5% reaction volume will interfere with the fluorescent total dsDNA quantification). At this volume of swab extract, DNA concentration is likely to be within the linear range of both the QuantiFluor® dsDNA System and Quant-iT™ PicoGreen® dsDNA Reagent.

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7. Related Products

Product	Size	Cat.#
PunchSolution™ Kit*	100 preps	DC9271
5X AmpSolution™ Reagent*	500µl	DM1231
Plexor® HY System*	200 reactions	DC1001
	800 reactions	DC1000
PowerQuant® System	200 reactions	PQ5002
	800 reactions	PQ5008
ClickFit Microtube, 1.5ml	100/pack	V4745
2.2ml, Square-Well Deep Well Plate	50/case	V6781
Heat Block Adapter	1 each	A2661
QuantiFluor® dsDNA System	1ml	E2670

*Not for Medical Diagnostic Use.

8. Summary of Changes

The following changes were made to the 4/21 revision of this document:

1. Inserted new Section 6.A.
2. Revised Section 7.
3. Updated cover page.