Rapid Processing of Swabs from Casework Samples Using Casework Direct Kit, Custom

1. Description

The Casework Direct Kit, Custom, is used for rapid processing of swabs from casework samples, cuttings of sexual assault swabs or cuttings of stained fabric prior to quantification of human DNA using either the PowerQuant® System or Plexor® HY System and amplification of normalized template using PowerPlex® Systems for human STR genotyping. For casework samples no subsequent purification of the Casework Direct lysate is required prior to STR amplification unless data from the PowerQuant® or Plexor® HY System indicates the presence of possible PCR inhibitors. In this case, sample cleanup may be performed using the DNA IQ™ Casework Pro Kit for Maxwell® 16 (Cat.# AS1240) on the Maxwell® 16 Forensic Instrument (Cat.# AS3060), or the Maxwell® FSC DNA IQ™ Casework Kit (Cat.# AS1550) on the Maxwell® FSC Instrument (Cat.# AS4600) or the Maxwell® RSC 48 Instrument (Cat.# AS8500). Sample processing decisions in Y-screening applications are facilitated by the Y quantification result and Auto/Y ratio. If desired, the Casework Direct lysate may be used directly in STR amplification (e.g., for amplification with the PowerPlex® Y23 System if the Auto/Y ratio indicates very low level male contributor DNA). The Casework Direct Kit, Custom, contains Casework Direct Reagent, Custom, and 1-Thioglycerol as a reducing agent. In addition, the Casework Direct Kit, Custom, contains 5X AmpSolution™ Reagent, which must be added to Plexor® HY System amplification reactions and certain PowerPlex® Systems when adding the maximum volume of Casework Direct lysate to the amplification reaction (see Table 1). A developmental validation study of the use of Casework Direct Kit, Custom with forensic casework sample was performed and the results are documented in a white paper (1).

Table 1. 5X AmpSolution™ Reagent Requirements for Casework Direct Solution. Final concentration of AmpSolution™ Reagent in STR amplification reaction mixes is 0.5X.

PowerPlex® System	Maximum Sample Volume Available	Maximum Sample Volume in Absence of 5X AmpSolution™ Reagent	Maximum Sample Volume in Presence of 5X AmpSolution™ Reagent	Volume of 5X AmpSolution™ Reagent
PowerPlex® ESX Fast and ESI Fast	17.5µl	NA	15µl	2.5µl
PowerPlex® Fusion	15µl	15µl	NA	NA
PowerPlex® Fusion 6C	15µl	15µl	NA	NA
PowerPlex® Y23	17.5µl*	15µl	NA	NA
PowerPlex® 21	15µl	15µl	NA	NA

*For the PowerPlex® Y23 System it is possible to add sample volumes of 17.5µl to the standard 25µl amplification reaction as shown. However, if Casework Direct lysate is added, the volume should not exceed 15µl; above this volume inhibition may be seen.

2. Product Components and Storage Conditions

Please contact your Promega representative to order the Casework Direct Kit, Custom. Contact information available at: **www.promega.com**. E-mail: **genetic@promega.com**

PRODUCT	SIZE
Casework Direct Kit, Custom	1 each

Not For Medical Diagnostic Use.

Includes:

- 100ml Casework Direct Reagent, Custom
- 75µl 1-Thioglycerol
- 500µl 5X AmpSolution™ Reagent
- 5 x 1,250µl Water, Amplification Grade

The 5X AmpSolution™ Reagent is used for amplification of Casework Direct Solution lysates with certain PowerPlex® Systems (see Table 1) and Plexor® HY System.

Storage Conditions: Upon arrival, thaw Casework Direct Reagent completely by placing in a 4°C refrigerator overnight. After thawing, mix thoroughly by gentle inversion and store at 2–10°C. Thaw 1-Thioglycerol and Water, Amplification Grade, and store at 2–10°C. Thaw 5X AmpSolution™ Reagent completely (in a 37°C water bath or at ambient temperature) and mix by vortexing before storing at 2–10°C. The 5X AmpSolution™ Reagent may be turbid after thawing or storage at 4°C. If turbidity occurs, warm the reagent briefly at 37°C, and 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.

3. Processing Swabs and Cuttings from Stained Fabric

Materials to Be Supplied by the User

- ClickFit Microtubes (Cat.# V4745)
- CW Spin Baskets (Cat.# AS8101)
- aerosol-resistant pipette tips
- heat block for 1.5ml tubes

Note: Do not use an incubator to incubate tubes. Heat transfer is inefficient and will result in poor performance. Only use a heat block to maintain efficient heat transfer. For long term storage of samples, we recommend the use of ClickFit tubes.

3.A. Preparation of Casework Direct Solution

Dilute 1-Thioglycerol tenfold by adding 50µl of 1-Thioglycerol to 450µl of Water, Amplification Grade. Vortex tube for 10–15 seconds. The diluted 1-Thioglycerol is stable at 4°C for 6 months.

Note: 1-Thioglycerol is viscous. To facilitate accurate pipetting, warm to room temperature, pipette slowly and avoid pipetting small volumes.

Add diluted 1-Thioglycerol to Casework Direct Reagent to make Casework Direct Solution as shown in Table 2.

Table 2. Casework Direct Solution.

Mix Component	Volume Per Extraction	×	Number of Extractions	=	Final Volume
Casework Direct Reagent	400µl	×		=	
1-Thioglycerol (diluted tenfold)	2µl	×		=	

Mix Component	Volume Per Extraction	×	Number of Extractions	=	Final Volume
Casework Direct Reagent	200µl	×		=	
1-Thioglycerol (diluted tenfold)	1µl	×		=	

Mix Component	Volume Per Extraction	×	Number of Extractions	=	Final Volume
Casework Direct Reagent	100µl	×		=	
1-Thioglycerol (diluted tenfold)	0.5µl	×		=	

Note: The volume of Casework Direct Solution used per sample depends on swab or cutting size. We have successfully tested volumes from 100–400µl. The larger the swab or cutting, the larger the volume of Casework Direct Solution that is required to completely submerge the swab or cutting. Do not exceed 400µl; using volumes less than 400ul may reduce the volume of lysate recovered from the CW Spin Basket. See Troubleshooting (Section 9) for additional information.

3.B. Processing of Swab/Stained Fabric Cutting

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 the CW Spin Basket inside a ClickFit Microtube, 1.5ml.
3. Place swab head, cutting of swab, cutting of a sexual assault swab or fabric cutting inside the CW Spin Basket.
4. Add 100–400µl of Casework Direct Solution to each swab or cutting. Close the lid of the CW Spin Basket/ ClickFit Microtube assembly. Vortex tube for 5–10 seconds.
5. Place tube in heat block, and incubate sample at 70°C for 30 minutes.
6. Vortex for 5–10 seconds, and then centrifuge at room temperature for 5 minutes at maximum speed in a microcentrifuge. Carefully remove the CW Spin Basket containing the swab or cutting. Close the tube.

4. Quantification of Human DNA with the PowerQuant® System

The amount of human genomic DNA present in the lysate may be quantified using the PowerQuant® System. Addition of 5X AmpSolution™ Reagent to PowerQuant® amplification reactions is not required for quantification using the PowerQuant® System. Follow the *PowerQuant® System Technical Manual* #TMD047, without any changes to this protocol, using 2µl of lysate per 20µl PowerQuant® System amplification reaction. In addition, the quality of human DNA in the lysate may be evaluated using the PowerQuant® System 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® System IPC target can indicate whether PCR inhibitors are present, which are likely to inhibit downstream STR amplification. If inhibitors are indicated, we recommend that the lysate be cleaned up using the Maxwell® FSC DNA IQ™ Casework Kit following the protocol for liquid samples in the *Maxwell® FSC DNA IQ Casework Kit Technical Manual* #TM499 (Section 3.B, Step 4) or the DNA IQ™ Casework Pro Kit for Maxwell® 16 following the protocol for liquid samples in the *DNA IQ™ Casework Pro Kit for Maxwell® 16 Technical Manual* #TM332 (Section 4.B, Step 3). Use up to a maximum of 400µl of lysate per purification.

5. Quantification of Human DNA with the Plexor® HY System

The Plexor® HY System has an absolute requirement for 5X AmpSolution™ Reagent to be able to tolerate 2µl of extract per 20µl Plexor® HY System amplification reaction (Table 3). In addition to quantification, the ratio of the autosomal to Y target quantification results can provide information on the ratio of female to male DNA in the sample, whereas the Plexor® HY IPC target can indicate whether or not PCR inhibitors are present that are likely to inhibit downstream STR amplification. If inhibitors are indicated it is recommended that the extracts be cleaned up using the Maxwell® FSC DNA IQ™ Casework Kit following the protocol for liquid samples in the *Maxwell® FSC DNA IQ Casework Kit Technical Manual* #TM499 (Section 3.B, Step 4) or the DNA IQ™ Casework Pro Kit for Maxwell® 16 following the recommended protocol for liquid samples in the *DNA IQ™ Casework Pro Kit for Maxwell® 16 Technical Manual* #TM332 (Section 4.B, Step 3). Use up to a maximum of 400µl of lysate per purification.

Table 3. PCR Amplification Mix for Plexor® HY System with Casework Direct Solution Lysate.

PCR Amplification Mix Component	Volume Per Reaction	×	Number of Reactions	=	Final Volume
Plexor® HY 2X Master Mix	10µl	×		=	
Plexor® HY 20X Primer/IPC Mix	1µl	×		=	
5X AmpSolution™ Reagent	4µl	×		=	
Water, Amplification Grade	3µl	×		=	
Total Reaction Mix Volume	18µl	×		=	

Note: Final concentration of AmpSolution™ Reagent in amplification reaction mix is 1X.

Add 2µl of Casework Direct Solution lysate to 18µl of amplification reaction mix.

Perform thermal cycling as described in the technical manual for the Plexor® HY System.

6. Amplification with PowerPlex® Systems Requiring 5X AmpSolution™ Reagent

The PowerPlex® ESI Fast and ESX Fast Systems require 5X AmpSolution™ Reagent for efficient amplification in the presence of Casework Direct Solution (see Table 4).

Table 4. PCR Amplification Mix for PowerPlex® ESI Fast and ESX Fast Systems with Casework Direct Solution Lysate.

PCR Amplification Mix Component	Volume Per Reaction	×	Number of Reactions	=	Final Volume
PowerPlex® ESI/ESX Fast 5X Master Mix	5µl	×		=	
PowerPlex® ESI 16/17 or ESX 16/17 Fast 10X Primer Pair Mix	2.5µl	×		=	
5X AmpSolution™ Reagent	2.5µl	×		=	
Total Reaction Mix Volume	10µl	×		=	

Note: Final concentration of AmpSolution™ Reagent in amplification reaction mix is 0.5X.

Add up to 15µl of Casework Direct Solution lysate to 10µl of amplification reaction mix.

Perform thermal cycling and subsequent analysis of amplified products by capillary electrophoresis as described in the technical manuals for the PowerPlex® ESI Fast or ESX Fast System, as appropriate.

7. Amplification with PowerPlex® Systems Not Requiring 5X AmpSolution™ Reagent

The PowerPlex® Fusion, Fusion 6C and 21 Systems do not require 5X AmpSolution™ Reagent. Set up the reactions for each PowerPlex® System in the same way using 5X Master Mix and 5X Primer Pair Mix. PowerPlex® Fusion 6C is shown below as an example (see Table 5).

Table 5. PCR Amplification Mix for PowerPlex® Fusion 6C System with Casework Direct Solution Lysate.

PCR Amplification Mix Component	Volume Per Reaction	×	Number of Reactions	=	Final Volume
PowerPlex® Fusion 6C 5X Master Mix	5µl	×		=	
PowerPlex® Fusion 6C 5X Primer Pair Mix	5µl	×		=	
Total Reaction Mix Volume	10µl	×		=	

Add up to 15µl of Casework Direct Solution lysate to 10µl of amplification reaction mix.

Perform thermal cycling and subsequent analysis of amplified products by capillary electrophoresis as described in the technical manuals for the PowerPlex® Fusion, Fusion 6C or 21 System, as appropriate.

8. Amplification with the PowerPlex® Y23 System

The PowerPlex® Y23 System does not require 5X AmpSolution™ Reagent for volumes of Casework Direct Solution up to 15µl per 25µl amplification reaction. We do not recommend adding more than 15µl of Casework Direct Solution because of the potential for inhibition that can be seen at the maximum sample volume of 17.5µl (see Table 6).

Table 6. PCR Amplification Mix for PowerPlex® Y23 System with Casework Direct Solution Lysate.

PCR Amplification Mix Component	Volume Per Reaction	×	Number of Reactions	=	Final Volume
PowerPlex® Y23 5X Master Mix	5µl	×		=	
PowerPlex® Y23 10X Primer Pair Mix	2.5µl	×		=	
Water, Amplification Grade	2.5µl	×		=	
Total Reaction Mix Volume	10µl	×		=	

Add up to 15µl of Casework Direct Solution lysate to 10µl of amplification reaction mix.

Perform thermal cycling and subsequent analysis of amplified products by capillary electrophoresis as described in the technical manual for the PowerPlex® Y23 System.

9. Troubleshooting

Symptom	Causes and Comments
Very high degradation quantification value (e.g., >10,000ng/µl) for extract with PowerQuant® System, resulting in Auto/Deg ratio of 0.00.	The concentration of 1-Thioglycerol in the Casework Direct Solution is too high, resulting in artificially raised signal for the degradation target in the PowerQuant® System that crosses the amplification threshold prematurely (giving an artificially elevated estimate of DNA concentration). Ensure that you are adding diluted (tenfold) 1-Thioglycerol to the Casework Direct Reagent. When diluting 1-Thioglycerol tenfold with Water, Amplification Grade, ensure that you are pipetting large volumes of 1-Thioglycerol to increase accuracy. Also ensure that 1-Thioglycerol is not on the outside of the pipette, which results in an increased volume of concentrated 1-Thioglycerol added to the Water, Amplification Grade.
Liquid remains in the spin basket following centrifugation.	Centrifuge for an additional 5 minutes. Recovery of lysate from the spin basket can be dependent on the weight of the substrate and reagent. Inconsistency in recovered lystate has been occasionally observed for volumes <400uL and smaller cutting sizes.

10. References

1. Graham, E.K. *et al.* (2018) Developmental Validation of the Casework Direct Kit, Custom: A Method for the Rapid Processing of Casework Samples. Promega Corporation.

Please contact your Promega representative to order the Casework Direct Kit, Custom.
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