February 2019

QIAsymphony® SP Protocol Sheet

Casework_200_HE_V9 and Casework_200_H2O_HE_V9

This document is the CW_200_HE_V9 and CW_200_H2O_HE_V9 *QIAsymphony SP Protocol Sheet*, R1, for QIAsymphony DNA Investigator Kit.

General information

The QIAsymphony DNA Investigator Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

These protocols are for purification of total DNA from samples encountered in forensic, human identity, and biosecurity applications using the QIAsymphony SP and the QIAsymphony DNA Investigator Kit.

Since the type of samples that can be processed using the QIAsymphony DNA Investigator Kit can vary greatly, there is also a variety of different pretreatments, optimized for specific sample types. For the CW_200_HE_V9 and CW_200_H2O_HE_V9 protocol, samples are lysed under denaturing conditions in the presence of proteinase K and Buffer ATL in a total volume of 200 µl.

The Elution can be performed either with the buffer ATE (CW_200_HE_V9) or with the buffer AVE (CW_200_H2O_HE_V9).

Note: It is the user's responsibility to validate performance using this combination for any procedures used in their laboratory.

CW_200_H2O_HE_V9

Kit	QIAsymphony DNA Investigator Kit
Sample material	Chewing gum, cigarette butts, nail clippings, hair, sexual assault specimens, blood (<10 µl), and saliva (<10 µl)
Protocol name	CW_200_H2O_HE_V9
Default Assay Control Set	ACS_CW_200_H2O_HE_V9
Elution volume	30 µl, 40 µl, 50 µl, 60 µl, 70 µl, 80 µl
Elution solution	Buffer AVE
Required software version	Version 5.0 or higher

CW_200_HE_V9 protocol

Kit	QIAsymphony DNA Investigator Kit
Sample material	Chewing gum, cigarette butts, nail clippings, hair, sexual assault specimens, blood (<10 µl), and saliva (<10 µl)
Protocol name	CW_200_HE_V9
Default Assay Control Set	ACS_CW_200_HE_V9
Elution volume	30 µl, 40 µl, 50 µl, 60 µl, 70 µl, 80 µl
Elution solution	Buffer ATE
Required software version	Version 5.0 or higher

Materials required but not provided

For all sample types

- TopElute Fluid (60 ml) (cat. no. 1055628)
- Vortexer
- Thermomixer or shaker-incubator

For chewing gum and cigarette butts

- Scissors or appropriate cutting device

For nail clippings and hair

- Scissors or appropriate cutting device
- Dithiothreitol (DTT),* 1 M aqueous solution

For sexual assault specimens

- Plastic swabs with cotton or Dacron® tips (Puritan® applicators with plastic shafts and cotton or Dacron tips are available from: Hardwood Products Company, **www.hwppuritan.com**, item nos. 25-806 1PC and 25-806 1PD; and from Daigger, **www.daigger.com**, cat. nos. EF22008D and EF22008DA). Nylon cytology brushes and other swab types may also be used.†

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

† This is not a complete list of suppliers and does not include many important vendors of biological supplies.

- Dithiothreitol (DTT),* 1 M aqueous solution
- Additional Buffer ATL (200 ml) (cat. no. 19076)
- Additional QIAGEN® Proteinase K (2 ml) (cat. no. 19131)
- Microcentrifuge

"Sample" drawer

Sample type	Lysates from chewing gum, cigarette butts, nail clippings, hair, sexual assault specimens, blood (<10 µl), and saliva (<10 µl)
Sample volume	200 µl
Primary sample tubes	See **www.qiagen.com/goto/qsdnainvestigator** for more information
Secondary sample tubes	See **www.qiagen.com/goto/qsdnainvestigator** for more information
Inserts	See **www.qiagen.com/goto/qsdnainvestigator** for more information
Other	n/a

n/a = not applicable.

"Reagents and Consumables" drawer

Position A1 and/or A2	Reagent cartridge (RC)
Position B1	TopElute Fluid
Tip rack holder 1–17	Disposable filter-tips, 200 µl
Tip rack holder 1–17	Disposable filter-tips, 1500 µl
Unit box holder 1–4	Unit boxes containing sample prep cartridges
Unit box holder 1–4	Unit boxes containing 8-Rod Covers

"Waste" drawer

Unit box holder 1–4	Empty unit boxes
Waste bag holder	Waste bag
Liquid waste bottle holder	Liquid waste bottle

"Eluate" drawer

Elution rack (we recommend using slot 1, cooling position)	See **www.qiagen.com/goto/qsdnainvestigator** for more information

Required plasticware

	One batch, 24 samples*	Four batches, 96 samples*
Disposable filter-tips, 200 µl†‡	4	16
Disposable filter-tips, 1500 µl†‡	80	320
Sample prep cartridges§	15	60
8-Rod Covers¶	3	12

* Use of less than 24 samples per batch decreases the number of disposable filter-tips required per run.
† There are 32 filter-tips/tip rack.
‡ Number of required filter-tips includes filter-tips for 1 inventory scan per reagent cartridge.
§ There are 28 sample prep cartridges/unit box.
¶ There are twelve 8-Rod Covers/unit box.

Note: Numbers of filter-tips given may differ from the numbers displayed in the touchscreen depending on settings, for example, number of internal controls used per batch.

Preparation of sample material

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

Important points before starting

- QIAsymphony magnetic particles copurify RNA and DNA if both are present in the sample. If RNA-free DNA is required, add RNase A to the sample in the step indicated in the respective pretreatment protocol.
- Before beginning the procedure, read "Important Notes", page 12 of the *QIAsymphony DNA Investigator Handbook*.

Chewing gum

This protocol is for isolation of total (genomic and mitochondrial) DNA from chewing gum. The pretreatment includes lysis of samples using proteinase K.

Things to do before starting

- Before using Buffer ATL, check that it does not contain a white precipitate. If necessary, incubate for 30 minutes at 70°C with gentle agitation.
- Set a thermomixer or shaker–incubator to 56°C for use in step 4.

Pretreatment protocol for chewing gum

1. Cut up to 30 mg of chewing gum into small pieces and transfer them to a 1.5 ml or 2 ml microcentrifuge tube (not provided).
2. Add 180 µl Buffer ATL.
3. Add 20 µl proteinase K, and mix by vortexing.
4. Place the tube in a thermomixer or heated orbital incubator, and incubate with shaking at 900 rpm at 56°C for 15 min.
5. Carefully transfer the lysate to sample tubes or plates that are compatible with the sample rack of the QIAsymphony SP.

   See **www.qiagen.com/QIAsymphony/Resources** for a full list of compatible vessels. We recommend using 2 ml tubes (e.g., Sarstedt, cat. no. 72.693 or 72.608) or S-Blocks (cat. no. 19585).

   Note: Do not transfer any solid material as this may clog the tips during automated DNA purification.
6. Continue with the protocol "DNA Purification from Casework and Reference Samples" (page 19 in the *QIAsymphony DNA Investigator Handbook*).

Cigarette butts

This protocol is for isolation of total (genomic and mitochondrial) DNA from cigarette butts. The pretreatment includes lysis of samples using proteinase K.

Things to do before starting

- Before using Buffer ATL, check that it does not contain a white precipitate. If necessary, incubate for 30 minutes at 70°C with gentle agitation.
- Set a thermomixer or shaker–incubator to 56°C for use in step 4.

Pretreatment protocol for cigarette butts

1. Remove 1 cm^2 of the outer paper from the end of the cigarette or filter. Cut the piece into six smaller pieces and transfer to a 1.5 ml or 2 ml microcentrifuge tube (not provided).
2. Add 180 µl Buffer ATL.
3. Add 20 µl proteinase K, and mix by vortexing.
4. Place the tube in a thermomixer or heated orbital incubator, and incubate with shaking at 900 rpm at 56°C for 15 min.

5. Carefully transfer the lysate to sample tubes or plates that are compatible with the sample rack of the QIAsymphony SP.

   See **www.qiagen.com/QIAsymphony/Resources** for a full list of compatible vessels. We recommend using 2 ml tubes (e.g., Sarstedt, cat. no. 72.693 or 72.608) or S-Blocks (cat. no. 19585).

   Note: Do not transfer any solid material as this may clog the tips during automated DNA purification.

6. Continue with the protocol "DNA Purification from Casework and Reference Samples" (page 19 in the *QIAsymphony DNA Investigator Handbook*).

Nail clippings and hair

This protocol is for isolation of total (genomic and mitochondrial) DNA from nail clippings or hair. The pretreatment includes lysis of samples using proteinase K and DTT.*

Things to do before starting

- Before using Buffer ATL, check that it does not contain a white precipitate. If necessary, incubate for 30 minutes at 70°C with gentle agitation.
- Set a thermomixer or shaker–incubator to 56°C for use in step 4.
- Prepare an aqueous 1 M DTT stock solution. Store aliquots at –20°C. Thaw immediately before use.

Pretreatment protocol for nail clippings and hair

1. Cut nail clippings or hairs to 0.5–1 cm lengths, and transfer to a 1.5 ml or 2 ml microcentrifuge tube (not provided).
2. Add 160 µl Buffer ATL.
3. Add 20 µl proteinase K and 20 µl 1 M DTT. Mix by vortexing.
4. Place the tube in a thermomixer or heated orbital incubator, and incubate with shaking at 900 rpm at 56°C for 1 h.

   In general, samples are lysed in 1 h. If necessary, increase the incubation time to ensure complete lysis.

5. Carefully transfer the lysate to sample tubes or plates that are compatible with the sample rack of the QIAsymphony SP.

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

See **www.qiagen.com/QIAsymphony/Resources** for a full list of compatible vessels. We recommend using 2 ml tubes (e.g., Sarstedt, cat. no. 72.693 or 72.608) or S-Blocks (cat. no. 19585).

Note: Do not transfer any solid material as this may clog the tips during automated DNA purification.

6. Continue with the protocol "DNA Purification from Casework and Reference Samples" (page 19 in the *QIAsymphony DNA Investigator Handbook*).■

Sexual assault specimens

This protocol is for differential extraction of total (genomic and mitochondrial) DNA from fabrics or swabs containing epithelial cells mixed with sperm cells. The pretreatment includes differential lysis of epithelial and sperm cells.

Things to do before starting

- Before using Buffer ATL, check that it does not contain a white precipitate. If necessary, incubate for 30 minutes at 70°C with gentle agitation.
- Set a thermomixer or shaker–incubator to 56°C for use in step 4.
- Prepare an aqueous 1 M DTT* stock solution. Store aliquots at –20°C. Thaw immediately before use.
- Additional Buffer ATL (200 ml) (cat. no 19076) and QIAGEN Proteinase K (2 ml) (cat. no. 19131) is required for this protocol and can be ordered separately.
- Optional: To harvest lysate remaining in the swab or stain, QIAshredder spin columns (50) (cat. no. 79654) may be required.

Pretreatment protocol for sexual assault specimens

1. Place the swab or the piece of fabric into a 2 ml microcentrifuge tube (not provided).

   If using an Omni Swab, eject the swab by pressing the end of the stem towards the swab.

   If using a cotton or Dacron swab, separate the swab from its shaft by hand or using scissors.
2. Add 475 µl Buffer ATL.
3. Add 25 µl proteinase K, and mix by vortexing.

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

4. Place the tube in a thermomixer or heated orbital incubator, and incubate with shaking at 900 rpm at 56°C for 1 h.
5. Remove the solid material from the tube.

   Lysate remaining in the swab or fabric can be harvested by transferring the material to a QIAshredder spin column (not supplied) and centrifuging at full speed for 2 min in a microcentrifuge. Remove and discard the QIAshredder spin column and the solid material.
6. Centrifuge the tube for 5 min at full speed in a microcentrifuge. Carefully transfer all but 30 µl of the supernatant to a new tube without disturbing the pellet.

   Note: For isolation of DNA from epithelial cells, transfer 200 µl of the supernatant into an appropriate sample tube and continue with the Casework_200_HE_V8 or Casework_200_H2O_HE_V8 protocol.
7. Resuspend the pellet in 500 µl Buffer ATL. Close the lid and mix by pulse-vortexing for 10 s. Centrifuge the tube for 5 min at full speed in a microcentrifuge. Carefully aspirate and discard all but 30 µl of the supernatant without disturbing the pellet.
8. Repeat step 7 for a total of at least 4 times.

   Note: The ratio of epithelial cells to sperm cells influences the number of repeats needed for purification of sperm nuclei.
9. Add 160 µl Buffer ATL, 20 µl proteinase K, and 20 µl 1 M DTT to the pellet. Mix by vortexing.
10. Place the tube in a thermomixer or heated orbital incubator, and incubate with shaking at 900 rpm at 56°C for at least 1 h.
11. Carefully transfer the lysate to sample tubes or plates that are compatible with the sample rack of the QIAsymphony SP.

    See **www.qiagen.com/QIAsymphony/Resources** for a full list of compatible vessels. We recommend using 2 ml tubes (e.g., Sarstedt, cat. no. 72.693 or 72.608) or S-Blocks (cat. no. 19585).

    Note: Do not transfer any solid material as this may clog the tips during automated DNA purification.
12. Continue with the protocol "DNA Purification from Casework and Reference Samples" (page 19 in the *QIAsymphony DNA Investigator Handbook*).

Blood and saliva (<10 µl)

This protocol is designed for isolation of total (genomic and mitochondrial) DNA from up to 10 µl of whole blood treated with EDTA,* citrate,* or heparin-based* anticoagulants or up to 10 µl of saliva. The pretreatment includes lysis of samples using proteinase K.

Things to do before starting

- Equilibrate samples to room temperature (15–25°C).
- Set a thermomixer or heated orbital incubator to 56°C for use in step 4.
- If Buffer ATL contains precipitates, dissolve by heating to 70°C with gentle agitation.

Pretreatment protocol for blood and saliva

1. Pipet up to 10 µl blood or saliva into a sample tube or plate that is compatible with the sample rack of the QIAsymphony SP.

   See **www.qiagen.com/QIAsymphony/Resources** for a full list of compatible vessels. We recommend using 2 ml tubes (e.g., Sarstedt, cat. no. 72.693 or 72.608) or S-Blocks (cat. no. 19585).
2. Add Buffer ATL to a final volume of 180 µl.
3. Add 20 µl proteinase K, and mix by vortexing.
4. Place the tube or plate into a thermomixer or heated orbital incubator and incubate with shaking at 900 rpm at 56°C for 10 min.
5. Continue with the protocol "DNA Purification from Casework and Reference Samples" (page 19 in the *QIAsymphony DNA Investigator Handbook*).

Revision history

Document revision history	
R1 02/2019	Update for QIAsymphony Software version 5.0

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN® kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

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