February 2019

# QIAsymphony® SP Protocol Sheet

## Casework_500_ADV_HE_V10 protocol

This document is the Casework_500_ADV_HE_V10 *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_500_ADV_HE_V10 protocol, samples are lysed under denaturing conditions in the presence of proteinase K and Buffer ATL in a total volume of 500 µl.

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

| | |
|---|---|
| **Kit** | QIAsymphony DNA Investigator Kit |
| **Sample material** | Surface swabs, body fluid stains, paper and similar materials, bones, and teeth |
| **Protocol name** | CW_500_ADV_HE_V10 |
| **Default Assay Control Set** | ACS_CW_500_ADV_HE_V10 |
| **Editable** | Elution volume: 30 µl, 40 µl, 50 µl, 60 µl, 70 µl, 80 µl |
| **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 surface swabs

- 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.*
- Microcentrifuge
- Scissors or appropriate cutting device
- Optional: QIAshredder spin columns (for maximum yields) (cat. no. 79654)

### For body fluid stains

- Optional: QIAshredder spin columns (for maximum yields) (cat. no. 79654)
- Optional: Dithiothreitol (DTT),† 1 M aqueous solution, for semen stains

### For paper and similar materials

- Scissors or appropriate cutting device

### For bones and teeth

- Metal blender (e.g., Waring*), or TissueLyser II with the Grinding Jar Set, S. Steel
- Liquid nitrogen†
- Microcentrifuge

## "Sample" drawer

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|---|---|
| **Sample type** | Lysates from surface swabs, body fluid stains, paper and similar materials, bones, and teeth |
| **Sample volume** | 500 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.

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

† 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.

## "Reagents and Consumables" drawer

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|---|---|
| **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

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

## "Eluate" drawer

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| **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*.

### Surface swabs

This protocol is for isolation of total (genomic and mitochondrial) DNA from surface, sperm, blood, and saliva swabs. 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.
- Let the swab air dry for at least 2 h after sample collection.
- If processing semen swabs, prepare an aqueous 1 M DTT* stock solution. Store aliquots at –20°C. Thaw immediately before use.
- Optional: To harvest lysate remaining in the swab, QIAshredder spin columns may be required.

### Pretreatment protocol for surface swabs

1. Place the swab into a 2 ml microcentrifuge tube (not provided).

   If using a Whatman® 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

* 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.

2. Add 475 µl Buffer ATL.

   Add 25 µl proteinase K, and mix by vortexing. If processing semen swabs, add 20 µl 1 M DTT.

3. Place the tube in a thermomixer or heated orbital incubator, and incubate with shaking at 900 rpm at 56°C for 15 min.

4. Briefly centrifuge the tube in a microcentrifuge to remove drops from the inside of the lid.

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.

   Lysate remaining in the swab 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. Transfer the flow-through to the sample tube.

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

## Body fluid stains

This protocol is for isolation of total (genomic and mitochondrial) DNA from material stained with blood, saliva, or semen. The pretreatment includes lysis of samples using proteinase K. For samples requiring larger lysis volumes, see the "Pretreatment of large-volume samples" protocol (see protocol sheets at **www.qiagen.com/goto/qsdnainvestigator**).

## 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.
- If processing semen stains, prepare an aqueous 1 M DTT* stock solution. Store aliquots at –20°C. Thaw immediately before use.
- Optional: To harvest lysate remaining in the swab, QIAshredder spin columns may be required.

* 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.

## Pretreatment protocol for body fluid stains

1. Place the stained material into a 2 ml microcentrifuge tube (not provided).
2. Add 475 µl Buffer ATL.
3. Add 25 µl proteinase K, and mix by vortexing. If processing semen stains, add 20 µl 1 M DTT.
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.

   Lysate remaining in the swab 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. Transfer the flow-through to the sample tube.
6. Continue with the protocol "DNA Purification from Casework and Reference Samples" (page 19 in the *QIAsymphony DNA Investigator Handbook*).

## Paper and similar materials

This protocol is for isolation of total (genomic and mitochondrial) DNA from paper evidence samples, such as saliva on envelope flaps and stamps or fingerprints on documents. The pretreatment includes lysis of samples using proteinase K. For samples requiring larger lysis volumes, see the "Pretreatment of Large-Volume Samples" protocol (see protocol sheets at **www.qiagen.com/goto/qsdnainvestigator**).

## 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 paper and similar materials

1. Remove a 0.5 to 2.5 $cm^2$ piece from the paper or similar material, and cut into smaller pieces. Transfer the pieces to a 2 ml microcentrifuge tube (not provided).
2. Add 475 µl Buffer ATL.
3. Add 25 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*).

## Bones and teeth

This protocol is for isolation of total (genomic and mitochondrial) DNA from pieces of bones and teeth. The pretreatment includes the mechanical disruption and lysis of samples. Lysis time will vary depending on the size and density of the source material. The lysis conditions given here are intended to serve as guidelines.

## 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 5.

Pretreatment protocol for bones and teeth

1. Crush the bone or teeth into small fragments. Grind to a fine powder using a metal blender half-filled with liquid nitrogen.* Alternatively, grind the bone or teeth to a fine powder using the TissueLyser II and the Grinding Jar Set, S. Steel.

   When using the TissueLyser II, transfer the bone or tooth sample and the ball into the grinding jar. Pour liquid nitrogen into the grinding jar over the ball and bone or tooth fragments. Allow the temperature to equilibrate (i.e., liquid nitrogen stops boiling). Decant the excess liquid nitrogen, close the grinding jar with the lid, and transfer it to the TissueLyser II. Grind the bone at 30 Hz for 1 min or until the sample is pulverized (grinding times depend on type, condition, and size of sample).

2. Transfer up to 100 mg of bone or teeth powder in a 1.5 ml or 2 ml microcentrifuge tube (not provided).

3. Add 475 µl Buffer ATL.

   Add 25 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 overnight.

5. Centrifuge the tube at full speed for 1 min in a microcentrifuge.

6. 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*).

* 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.

## Revision history

| Document revision history | |
|---|---|
| R1 02/2019 | New release |

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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