



Automated DNA Extraction



PDQeX Standard - DNA Extraction  
(for use with PDQeX XBU- and XBC- kits)

... simple

# Swab DNA extraction protocol

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## Sample Types:

- Buccal swabs
- Cigarettes
- Saliva and saliva stains.
- Mucosal swabs (throat, vagina etc.)
- Tissue culture and suspended cells (1000 -  $10^5$  cells)

## Reagent Storage:

- *prepGEM* or *forensicGEM*            -20°C
- BLUE Buffer                                    4°C

## Method:

1. Completely thaw *forensicGEM* reagent and mix by gently inverting the tube repeatedly. Remove BLUE buffer from refrigerator and mix.
2. Prepare a master mix for extraction. Per 100  $\mu$ l extraction:  

10 $\mu$ l	BLUE Buffer
2 $\mu$ l	<i>prepGEM</i> or <i>forensicGEM</i>
DNA-free water to 100 $\mu$ l	
3. Dispense 100  $\mu$ l of master mix into each PDQeX cartridge. Flick the liquid to the bottom of the tube ensuring there are no bubbles.
4. Add the sample, making sure that it is completely submerged in the reagents.

Sample types include:

- Portion of a buccal or mucosal swab
- Cell suspension
- Strip of paper from a cigarette butt
- Liquid saliva (up to 20  $\mu$ l)
- For larger swabs, it is sometimes a preferred method to wash the swab in 400  $\mu$ l of water and use 20 to 40  $\mu$ l of the wash in the extraction.

5. Put the cap on the PDQeX cartridge by inserting the tapered peg into the cartridge.
6. Load a 24-well plate or 8-strip tubes in the collection drawer and put the drawer in place.
7. Insert the PDQeX cartridges into the heating block.

**MAKE SURE THE PDQeX CARTRIDGES CORRESPOND WITH A COLLECTION TUBE OR WELL**

8. Cover the cartridge with the hinged flap and close the sliding door.
9. Select the "Buccal Swab" program.

Default program:

75° C	5 mins.
110° C	2 mins.

- Times may be adjusted by internal laboratory validations.
- Changes to the default temperatures are not recommended.

## PRECAUTIONS

1. Do not load the machine if the control screen indicates a temperature above 50°C.
2. Open the sliding door, and ensure the collection drawer and heating block are clean and DNA-free.
3. Ensure the collection drawer is inserted as far as possible, and that it is straight.
4. If less than 24 reactions are planned, ensure that the PCR tubes are placed in the holes in the drawer corresponding to the channels to be used in the heating block.

# Blood Protocol

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**NOTE:** The PDQeX Standard System is not designed to produce pure DNA from Blood. If you require high quality DNA, please use the PDQeX Blood kit.

In some circumstances, the PDQeX Standard provides a cost-saving option for blood when purity is not important.

## Sample Types:

- Liquid Blood (Fresh, Heparin, EDTA, Citrate)
- Blood stains - direct extraction from fabric
- Lifted blood swabs

## Reagent Storage:

- *prepGEM* or *forensicGEM*            -20°C
- **RED** Plus Buffer                            4°C

## Method:

1. Completely thaw *forensicGEM* and mix by gently inverting the tube repeatedly. Remove **RED** Plus buffer from refrigerator and mix.
2. Prepare a master mix for blood lysis. Per 100 µl extraction:

10 µl	<b>RED</b> Plus Buffer
86 µl	DNA-free water (not supplied)
2 µl	<i>prepGEM</i> or <i>forensicGEM</i>
3. Dispense 100 µl of master mix into each PDQeX cartridge. Flick the liquid to the bottom of the tube ensuring there are no bubbles.
4. Add the sample (1-2 µl of liquid blood), making sure that it is completely mixed into the reagents.
5. For swabs, add 1/4 of the swab head and for fabric, a 5 mm x 5 mm piece is usually sufficient.

6. Put the cap on the PDQeX cartridge by inserting the tapered peg into the cartridge.
7. Load a 24-well plate or 8-strip tubes in the collection drawer and put the drawer in place.
8. Insert the PDQeX cartridges into the heating block.

**MAKE SURE THE PDQeX CARTRIDGES CORRESPOND WITH A COLLECTION TUBE OR WELL**

9. Cover the tubes with the hinged flap and close the sliding door.
10. Select the "Blood Standard" program.

#### Default Blood Standard program:

75° C	5 mins.
95° C	5 mins.
110° C	2 mins.

- Times may be adjusted by internal laboratory validations.
- Changes to the default temperatures are not recommended.

#### PRECAUTIONS

1. Do not load the machine if the control screen indicates a temperature above 50°C.
2. Open the sliding door, and ensure the collection drawer and heating block are clean and DNA-free.
3. Ensure the collection drawer is inserted as far as possible, and that it is straight.
4. If less than 24 reactions are planned, ensure that the PCR cartridges are placed in the holes in the drawer corresponding to the channels to be used in the heating block.

## Technical tips for sample management

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- The device must be kept clean and DNA-free. The UV cycle will assist in removing DNA but the wells and the top and base of the heating block should be cleaned regularly using dilute HClO bleach on a swab. Wipe residual bleach off the surfaces using a second swab soaked with DNA-free water.
- The device creates DNA that can be used for SNIps, STRs, quantitative, multiplex and routine PCR applications.
- OD<sub>260</sub> methods for yield estimation are unsuitable for the DNA produced by the PDQeX.
- For accurate yield assessment, a qPCR is recommended. The DNA produced by the PDQeX is approximately 90% single-stranded. If standard fluorescent chelating dyes are to be used for quantification, then this factor should be taken into consideration.
- For best results prepare and manage samples at 4°C, or on ice before and after extraction.
- For long term storage of the extracted DNA, add TE buffer to 1x and store at -20°C.

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