MicroGEM Quick-Start Guide

DNA Extraction Using

PDQeX

*prep*GEM Universal

MicroGEM

Going to extremes for
effortless DNA extraction

Find more information at
www.microgembio.com

or email
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QSG_005_190531_PDQeX prepGEM Universal

About this Guide

This Quickstart Guide provides methods to extract DNA from a range of sample types. We are available to provide help with developing any specific method for custom substrates. Please contact us at **info@microgembio.com.**

Procedure overview: MicroGEM extraction products use a unique mixture of thermophilic and mesophilic enzymes. If a 52°C step is included in the method, this is to allow the mesophilic enzyme cocktail *Histosolv* to weaken and degrade cellular material. The 75°C step then activates a thermophilic proteinase that lyses the cells, kills nucleases and strips the DNA of nucleosomes. A final 95°C step shrinks the PDQeX inner tubing, bursts the valve and pushes the extract through a column which removes the proteinase and inhibitors.

Preparation and Storage of Liquid Reagents

- *Histosolv* is a mixture of reagents that aids with digestion. It is delivered as a lyophilised powder. This should be resuspended in DNA-free water as follows:

Kit size (Rxn)	Code	Volume of water to add
50	XPU0050	0.55 ml
100	XPU0100	1.1 ml
500	XPU0500	5.5 ml
1000	XPU1000	11.0 ml

Reagent storage: *prep*GEM reagents are stable at room temperature but on arrival should be stored at 4°C. After tubes have been opened, the *prep*GEM enzyme and the *Histosolv* should be placed at -20°C to safeguard against accidental contamination. All buffers can remain at 4°C for convenience.

General Instructions

- All manipulations should be performed in a clean-room or a PCR hood.
- Labcoats, gloves and hairnets should be worn at all times.
- Use only certified DNA-free tubes and reagents.
- Wash equipment that will come into contact with the sample in 0.5% bleach. Rinse with DNA-free water.

Precautions

- Do not load the PDQeX machine if the control screen indicates a temperature above 35° C.
- Ensure the collection drawer and heating block are clean and DNA-free.
- Ensure the collection drawer is inserted as far as possible, and that it is straight.
- If fewer than 24 reactions are planned, make sure that the PCR tubes are placed in drawer wells corresponding to the channels to be used in the heating block.

MicroGEM Reagent QC

MicroGEM reagents are manufactured and quality tested following ISO 18385:2016. Our reagents are made from certified DNA-free chemicals and solutions, and all buffers and enzymes are treated with DNAse and UV before shipment.

Important Technical Tips

- PDQeX *prep*GEM Universal is a method that lyses cells and removes nucleoproteins from the DNA.
- There is no concentration step in the procedure and so the concentration of the extract is dependent on: 1) The quality of the sample; 2) In the case of swabs, the type of swab and the volume of water used to wash the swab; 3) The extraction volume (which in some cases can be scaled).
- DNA extracted using the PDQeX *prep*GEM Universal kit is suitable for High-Throughput Sequencing (HTS) and many types of genotyping including SNP analysis as well as quantitative, multiplex and end-point PCR.
- DNA extracted using PDQeX *prep*GEM Universal can be quantified using a qPCR or by using fluorescent dyes like Pico Green, iQuant, Qubit assays or the like. Nanodrop is incompatible with PDQeX reagents.
- As with any preparative method for nucleic acid extraction, best results are obtained when samples are handled at 4°C, or on ice, before and after extraction.
- The haem colouration from blood extractions carries through to the DNA leaving the sample slightly pink. This does not cause inhibition of PCR, qPCR or human profiling.
- For long term storage of the extracted DNA, add TE buffer to 1x (10 mM Tris, pH 7.5, 1 mM EDTA) and store at -20°C.

Buccal - Liquid, Swabs and Stains

The processing of the sample will vary dependent on sample type. For liquid samples, volumes should be between 5-20 µl. With cotton swabs, add 1/4 of the swab directly to the PDQeX cartridge. Stained fabric can be swabbed or small portions added directly to the tube.

Preparing the extraction mixture

For each extraction, make up:

10 µl	10x **BLUE** Buffer
2 µl	*prep*GEM
	DNA-free water - Add to a final volume of 100 µl
	(Note - this will vary depending on whether you add any liquid with your sample).

PDQeX extraction

1. Add the extraction mix with sample into each PDQeX cartridge (100 µl).
2. Put the cap on the PDQeX cartridge by completely inserting the tapered column into the cartridge.
3. Load into the collection drawer either:
   - 24 well plate
   - 8 strip tubes
   - Individual tubes
4. Put the drawer in place.
5. Insert the PDQeX cartridges into the heating block.
6. Cover the cartridges with the hinged flap and close the sliding door.

MAKE SURE THE PDQEX CARTRIDGES CORRESPOND WITH A COLLECTION TUBE OR WELL- OTHERWISE YOU WILL LOSE YOUR SAMPLE

7. Select the 'Buccal' program for the extraction:

75°C	5 mins
95°C	2 mins

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

Saliva on Storage Cards

Depending on the storage card, it is typical that the preservatives in the card are inhibitory to *Taq* DNA polymerase and so a pre-wash is recommended prior to DNA extraction. For storage cards without preservatives (e.g. MicroGEM Storage cards, Whatman 903), the pre-wash step is likely not necessary, and may potentially cause a loss of sample. Test a pre-wash step before running precious samples.

Pre-Wash (Optional)

1. Remove one 3 mm disc from the card-stored sample and place into a thin-walled PCR tube.
2. Wash the disk in 100 µl of DNA-free water by incubating at room temperature for 15 min.
3. Aspirate the water from the disc and discard the water.

Preparing the extraction mixture

For each extraction, make up:

10 µl	10x **BLUE** Buffer
2 µl	*prep*GEM
88 µl	DNA-free water

PDQeX extraction

1. Add the extraction mix with sample into each PDQeX cartridge (100 µl).
2. Put the cap on the PDQeX cartridge by completely inserting the tapered column into the cartridge.
3. Load into the collection drawer either:
    - 24 well plate
    - 8 strip tubes
    - Individual tubes
4. Put the drawer in place
5. Insert the PDQeX cartridges into the heating block.
6. Cover the cartridges with the hinged flap and close the sliding door.

MAKE SURE THE PDQEX CARTRIDGES CORRESPOND WITH A COLLECTION TUBE OR WELL- OTHERWISE YOU WILL LOSE YOUR SAMPLE

7. Select the 'Buccal' program for the extraction:

75°C	5 mins
95°C	2 mins

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

Solid Tissue

Cut or punch the tissue (fat, muscle, ear tags, scrappings, etc) into cubes of approximately 1- 2 mm^3.

Preparing the extraction mixture

For each extraction, make up:

10 µl	10x **ORANGE+** Buffer
2 µl	*prep*GEM
10 µl	*Histosolv*
78 µl	DNA-free water

PDQeX extraction

1. Add the extraction mix with sample into each PDQeX cartridge (100 µl).
2. Put the cap on the PDQeX cartridge by completely inserting the tapered column into the cartridge.
3. Load into the collection drawer either:
    - 24 well plate
    - 8 strip tubes
    - Individual tubes
4. Put the drawer in place.
5. Insert the PDQeX cartridges into the heating block.
6. Cover the cartridges with the hinged flap and close the sliding door.

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7. Select the 'Tissue' program for the extraction:

52°C	5 mins
75°C	10 mins
95°C	2 mins

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

Insects and Mouse/Rat Tails

Depending on the size of the insect, the whole insect can be crushed with a pipette tip during mixing, or a section of the insect (e.g. a leg) can be used. For mouse/rat tails, take a 1- 2 mm^3 cutting.

Preparing the extraction mixture

For each extraction, make up:

10 µl	10x **BLUE** Buffer
2 µl	*prep*GEM
88 µl	DNA-free water

PDQeX extraction

1. Add the extraction mix with sample into each PDQeX cartridge (100 µl).
2. Put the cap on the PDQeX cartridge by completely inserting the tapered column into the cartridge.
3. Load into the collection drawer either:
    - 24 well plate
    - 8 strip tubes
    - Individual tubes
4. Put the drawer in place.
5. Insert the PDQeX cartridges into the heating block.
6. Cover the cartridges with the hinged flap and close the sliding door.

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7. Select the 'Insect' program for the extraction:

75°C	15 mins
95°C	2 mins

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

Blood - Liquid, Swabs and Stains

The processing of the sample will vary dependent on sample type. For liquid samples (e.g. fresh, EDTA, heparin, citrate), volumes should be between 1-10 µl. With cotton swabs, add 1/4 of the swab directly to the PDQeX cartridge. Stained fabric can be swabbed or small portions added directly to the tube.

Preparing the extraction mixture

For each extraction, make up:

10 µl	10x **ORANGE+** Buffer
2 µl	*prep*GEM
10 µl	*Enhancer*
	DNA-free water - Add to a final volume of 100 µl (Note - this will vary depending on whether you add any liquid with your sample).

PDQeX extraction

1. Add the extraction mix with sample into each PDQeX cartridge (100 µl).
2. Put the cap on the PDQeX cartridge by completely inserting the tapered column into the cartridge.
3. Load into the collection drawer either:
    - 24 well plate
    - 8 strip tubes
    - Individual tubes
4. Put the drawer in place.
5. Insert the PDQeX cartridges into the heating block.
6. Cover the cartridges with the hinged flap and close the sliding door.

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7. Select the 'Blood' program for the extraction:

75°C	10 mins
95°C	2 mins
105°C	2 mins

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

Blood on Storage Cards

Depending on the storage card, it is typical that the preservatives in the card are inhibitory to *Taq* DNA polymerase and so a pre-wash is recommended prior to DNA extraction. For storage cards without preservatives (e.g. MicroGEM Storage cards, Whatman 903), the pre-wash step is likely not necessary, and may potentially cause a loss of sample. Test a pre-wash step before running precious samples.

Pre-Wash (Optional)

1. Remove one 3 mm disc from the card-stored sample and place into a thin-walled PCR tube.
2. Wash the disk in 100 µl of DNA-free water by incubating at room temperature for 15 min.
3. Aspirate the water from the disc and discard the water.

Preparing the extraction mixture

For each extraction, make up:

10 µl	10x **ORANGE+** Buffer
2 µl	*prep*GEM
10 µl	*Enhancer*
78 µl	DNA-free water

PDQeX extraction

1. Add the extraction mix with sample into each PDQeX cartridge (100 µl).
2. Put the cap on the PDQeX cartridge by completely inserting the tapered column into the cartridge.
3. Load into the collection drawer either:
    - 24 well plate
    - 8 strip tubes
    - Individual tubes
4. Put the drawer in place
5. Insert the PDQeX cartridges into the heating block.
6. Cover the cartridges with the hinged flap and close the sliding door.

MAKE SURE THE PDQEX CARTRIDGES CORRESPOND WITH A COLLECTION TUBE OR WELL- OTHERWISE YOU WILL LOSE YOUR SAMPLE

7. Select the 'Blood' program for the extraction:

75°C	10 mins
95°C	2 mins
105°C	2 mins

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

Tissue Culture

It is not recommended to scale the extraction volume below 100 µl. It is therefore advised to use >100,000 cells in your extraction. Wash cells in 1x PBS, pellet, and remove all of the liquid. The extraction mixture can be used directly to resuspend the cell pellet.

Preparing the extraction mixture

For each extraction, make up:

10 µl	10x **BLUE** Buffer
2 µl	*prep*GEM
88 µl	DNA-free water

PDQeX extraction

1. Add the extraction mix with sample into each PDQeX cartridge (100 µl).
2. Put the cap on the PDQeX cartridge by completely inserting the tapered column into the cartridge.
3. Load into the collection drawer either:
    - 24 well plate
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4. Put the drawer in place.
5. Insert the PDQeX cartridges into the heating block.
6. Cover the cartridges with the hinged flap and close the sliding door.

MAKE SURE THE PDQEX CARTRIDGES CORRESPOND WITH A COLLECTION TUBE OR WELL- OTHERWISE YOU WILL LOSE YOUR SAMPLE

7. Select the 'Buccal' program for the extraction:

75°C	5 mins
95°C	2 mins

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