ZyGEM

Automated DNA Extraction

Tissue DNA EXTRACTION

(for use with PDQeX XTI- and XTS- kits)

... simple

When your kit arrives

The Histosolv is delivered as a dry powder. When your kit arrives, add DNA-free water as follows.

Kit size (Rxn)	Code	Volume of water to add
100	XTI- XTS0100	1.1 ml
500	XTI- XTS0500	5.5 ml
1000	XTI- XTS1000	11.0 ml

Technical tips for sample management

- 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_{260} 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.

Animal tissue DNA Extraction Protocol

Sample Types:

- 1- 3 mm punches of animal tissue (muscle, fat etc)
- 1 - 5 hair follicles

Reagent Storage:

- Histosolv (Limit freeze/thaw cycles) -20°C
- *prep*GEM or *forensic*GEM -20°C
- ORANGE Plus Buffer 4°C

Method:

1. Completely thaw *forensic*GEM and Histosolv reagent and mix by gently inverting the tubes repeatedly. Remove ORANGE Plus buffer from refrigerator and mix.
2. Prepare a master mix for tissue lysis. Per 100 µl extraction:

10 µl	Histosolv
10 µl	ORANGE Plus Buffer
78 µl	DNA-free water (not supplied)
2 µl	*prep*GEM or *forensic*GEM

3. Dispense 100 µl of master mix into each PDQeX cartridge. Flick the liquid to the bottom ensuring there are no bubbles.
4. Add the sample, making sure that it is completely submerged in the reagents.
5. Put the cap on the PDQeX cartridge by inserting the tapered peg into the tube.
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 tubes with the hinged flap and close the sliding door.
9. Select the "Tissue" program.

Default Tissue program:

52°C	5 mins.
75° C	10 mins.
115° 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.

Insect DNA Extraction Protocol

Sample Types:

- Homogenised or crushed insects and insect parts

Note:

- The Histosolv is not needed with homogenised insects.

Reagent Storage:

- *prep*GEM -20°C
- BLUE Buffer 4°C

Method:

1. Completely thaw *prep*GEM. Remove BLUE buffer from refrigerator and mix.
2. Homogenise ~3mm^3 portion of insect in 88 µl of DNA-free water.
3. Add:

   10 µl BLUE Buffer
   2 µl *prep*GEM

4. Transfer to a PDQeX cartridge and flick the liquid to the bottom of the tube.
5. Put the cap on the PDQeX cartridge by inserting the tapered peg into the tube.
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 cartridges with the hinged flap and close the sliding door.
9. Use the following program.

75° C	15 mins.
115° C	2 mins.

- The 75°C incubation time may possibly be reduced for some samples.

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.

For more information, visit: www.zygem.com