InVivo Biosystems
Formerly NemaMetrix

Zebrafish Genotyping Kit

The Zebrafish Lysis Kit has been designed for straightforward, column-free extraction of PCR-ready DNA from *Danio rerio*. The kit contains a lysis and protease buffer system designed for rapid DNA extraction without the need for laborious and time consuming extraction methods. DNA extraction is performed in a single tube thereby reducing potential contamination and sample loss.

Extraction of DNA is simple, requiring the addition of 1 buffer & a 30 minute incubation before the DNA is ready for use directly in your PCR. Alternatively, it can be stored at -20°C for future use.

The DNA generated with the Zebrafish Lysis Kit is suitable for use in genotyping PCR reactions without further clean-up steps. Zebrafish lysate can be used with InVivo Biosystems' PCR Master Mix or other PCR reagents.

Components Included

Zebrafish Lysis Buffer A	1 x 50 mL
Zebrafish Lysis Buffer B	1 x 5 mL
PCR Master Mix	1 x 1 mL

Materials Required but not Included

Thermocycler	0.2 ml reaction tubes
Micropipettes	Vortex mixer
Mini-centrifuge	PCR grade dH2O
Lid Locks	

Shipping and Storage

Upon arrival the kit should be stored at -20°C. If stored correctly the kit will retain full activity for 12 months. The kit can go through 30 freeze/thaw cycles with no loss of activity.

Limitations of Product Use

The product may be used only for in vitro research purposes.

LYSIS PROCEDURE

General Procedure (single embryos & single fin clips):

1. Collect single embryo sample in a 0.2 mL strip tube and add 90µl *Zebrafish* ***Lysis Buffer A***. *(For volumes & instructions for fin clips refer to table below)*
2. Incubate the reaction at 95˚C for 30 min in the thermocycler or dry block heater.
3. After incubation, centrifuge strip tubes and add 10µL *Zebrafish* ***Lysis Buffer B*** to each tube and centrifuge again.
4. Use 2 µl immediately or store at -20°C.

Special Notes for Pooled Embryos:

1. Collect pooled embryo samples in a 0.75mL tube and add 1000µl *Zebrafish* ***Lysis Buffer A***.
2. Fit tube lids with 1.5mL tube lid locks to prevent tops from opening when heated. Incubate the reaction at 95˚C for 30 min in the thermocycler or dry block heater.
3. After incubation, centrifuge tubes and aliquot 180 µl of pooled embryo solution into 0.2mL strip tubes.
4. Add 10µL *Zebrafish* ***Lysis Buffer B*** to each 0.2mL tube and centrifuge again.
5. Use 2 µl immediately or store at -20°C.

Amount of Lysis Buffers to use with embryos

	Buffer A (ul)	Buffer B (ul)
Single Embryo	90	10
Single Finclip	180	20
Pooled Embryos*	1000	20
**use 0.75 mL tube*		

PCR PROCEDURE

1. Calculate necessary volumes of master mix, primers and water by multiplying samples & controls by component volumes. Add an additional 10% for error. Vortex then centrifuge solution. Refer to sample calculation below.
2. Combine 2x master mix, forward & reverse primer, and water in a 1mL tube. Centrifuge and use a pipette to distribute into 0.2mL tubes.
3. Add DNA sample from the lysis procedure into 0.2mL tubes and centrifuge to ensure the solution is mixed thoroughly.
4. Use a thermocycler to run PCR program.

Sample Calculation for 40 samples & a 20 µl reaction.

40 samples + 2 positive controls + 2 negative controls + 4 (10% error) = 48

	20 µl reaction	Total Volume Needed (µl)
2x Master Mix (µl)	10	480
Forward Primer (µl)	0.8	38.4
Reverse Primer (µl)	0.8	48.4
Water (µl)	6.4	307.2
DNA (µl)	2.0	96

Components for different volume PCR reactions:

	10µl	20µl	50µl	100µl
2x Master Mix (µl)	5	10	25	50
Forward Primer (µl)	0.4	0.8	2	4
Reverse Primer (µl)	0.4	0.8	2	4
Water (µl)	3.2	6.4	16	32
DNA (µl)	1	2	5	10

Still need help, please contact
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