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Dechorionation of zebrafish embryos with Pronase for metronidazole-mediated ß-cell ablation V.2

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We use this protocol and it's working

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Abstract

Summary:

Zebrafish embryos develop in their eggshell (the chorion) until hatching (~48-72 hours post fertilization). Because the chorion is impermeable to water, it needs to be removed prior to metronidazole treatment and β-cell ablation in Tg(*ins:NTR-mCherry*) transgenic zebrafish embryos. This can be achieved either with forceps or by enzymatic removal using Pronase. The earliest time of removal should be after completion of gastrulation to avoid bursting of the yolk and potential infections.

Diabetic Complication:

Neuropathy

Materials

MATERIALS

Pronase from Streptomyces griseus **Roche Catalog #**10165921001

Sea salt **Instant Ocean (Pet store)**

1-phenyl-2-thiourea (PTU) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**P7629

Reagent/Material	Quantity Required
Pronase from *Streptomyces griseus*	1 g
Sea salt	0.3 g/ liter
1-phenyl-2-thiourea (PTU)	0.003 %

Reagent Preparation:

Reagent 1: Embryo medium
Preparation: Add 0.03 % Instant Ocean salt (Pet store) into double distilled water.

Reagent 2: Pronase stock solution
Preparation: Make a 10 mg/ml stock solution (10 ml) of Pronase and add 1 ml each into ten 15 ml falcon tubes. These can be frozen at -20ºC until needed.

Reagent 3: Embryo medium with 1-phenyl-2-thiourea (PTU) to prevent pigment formation.
Preparation: To embryo medium in a small beaker add a final of 0.003% PTU and dissolve overnight using a magnetic stir bar. Keep PTU solution in the dark.

Note:

Roche, RRID:SCR_001326
Sigma-Aldrich, RRID:SCR_008988

Troubleshooting

Protocol:

1 For dechorionation, add 9 ml of embryo medium containing 0.003% PTU to 15 ml Falcon tubes containing 1 ml of the Pronase stock solution to make a 10 ml working solution of 1 mg/ml. Pour the working solution into a small petri dish and place the embryos still in their chorion into the solution. Swirl the embryos until the chorion becomes soft (tear off soft chorion with forceps under a stereomicroscope, typically after 3-5 minutes at 24 hours post fertilization). Immediately transfer the embryos into a petri dish with fresh embryo medium. Wash three times in fresh embryo medium.

Potential Pitfalls:

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1. The embryos do not survive after treatment: This could be if embryos were incubated too long in Pronase. Decrease the incubation time and increase the number of washes.

2. Dechorionation does not work: Pronase might be too old or animals are older than 24 hours post fertilization. Increase incubation time in Pronase until chorions become soft. This can be up to 10 minutes in 48 hpf larvae.