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Extracellular fluid extraction in zebrafish brain tissue and samples

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Fish behavior and physi...

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Maximino C, Puty B, Benzecry R, Araújo J, Lima MG, Batista EJO, Oliveira KRM, Crespo-Lopez ME, Herculano AM (2013). Role of serotonin in zebrafish (*Danio rerio*) anxiety: Relationship with serotonin levels and effect of buspirone, WAY 100635, SB 224289, fluoxetine and *para*-chlorophenylalanine (pCPA) in two behavioral models. *Neuropharmacology* 71: 83-97. https://doi.org/10.1016/j.neuropharm.2013.03.006

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We use this protocol and it's working

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Abstract

This protocol is used to extract the contents of the extracellular fluid of the adult zebrafish brain, allowing quantification of analytes such as neurotransmitter (e.g., Maximino et al., 2013) or proteins (e.g., Pradel et al., 1999).

Protocol materials

- Tris HCl **P212121**
- Tris Base **Fisher Scientific Catalog #**BP152-1
- Sodium chloride **P212121**
- Calcium Chloride
- Glutathione
- Magnesium sulfate heptahydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**M2773
- Glucose **P212121 Catalog #**Glucose
- Calcium Chloride
- Sodium bicarbonate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**S6014
- Sodium chloride **P212121**
- Potassium chloride **P212121**
- Potassium phosphate (monobasic) **P212121**

Troubleshooting

Reagent preparation

1 Prepare the extraction fluid. The recipe below is good for 1 L.

Citation

Maximino C, Puty B, Benzecry R, Araújo J, Lima MG, de Jesus Oliveira Batista E, Renata de Matos Oliveira K, Crespo-Lopez ME, Herculano AM
(2013)
. Role of serotonin in zebrafish (Danio rerio) anxiety: relationship with serotonin levels and effect of buspirone, WAY 100635, SB 224289, fluoxetine and para-chlorophenylalanine (pCPA) in two behavioral models..

https://doi.org/10.1016/j.neuropharm.2013.03.006 LINK

1.1 To make 1 L Tris buffer

- Add 4.44 g Tris HCl **P212121** to 1 L double distilled water
- Add 2.65 g Tris Base **Fisher Scientific Catalog #**BP152-1
- Check if pH 8.0 . If not, adjust with acid or base

1.2 To the Tris buffer, add 5.2596 g Sodium chloride **P212121**

1.3 Add 0.27745 g Calcium Chloride

1.4 Add 0.30733 g Glutathione

1.5 Adjust pH to pH 7.4

2 Prepare dissection solution (artificial cerebrospinal fluid). The recipe below is good for 1 L.

Citation

Vargas R, Jóhannesdóttir IT, Sigurgeirsson B, Thorsteinsson H, Karlsson KA (2011) . The zebrafish brain in research and teaching: a simple in vivo and in vitro model for the study of spontaneous neural activity..

https://doi.org/10.1152/advan.00099.2010 LINK

2.1
- Add 7.65564 g Sodium chloride **P212121** to 1 L double distilled water
- Add 0.1491026 g Potassium chloride **P212121**
- Add 0.1701 g Potassium phosphate (monobasic) **P212121**
- Add 0.240732 g Magnesium sulfate heptahydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**M2773
- Add 1.80156 g Glucose **P212121 Catalog #**Glucose
- Add 0.27745 g Calcium Chloride
- Add 1.68014 g Sodium bicarbonate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**S6014

2.2 Keep dissection solution on the fridge (4 ºC) for a maximum of 30 days before use.

Euthanasia and dissection

3 Sacrifice animals in ice-cold water.

Citation

Wallace CK, Bright LA, Marx JO, Andersen RP, Mullins MC, Carty AJ (2018)
. Effectiveness of Rapid Cooling as a Method of Euthanasia for Young Zebrafish (Danio rerio)..

https://www.ingentaconnect.com/content/aalas/jaalas/2018/00000057/00000001/art0
LINK

3.1 Add ice to a beaker filled with at least 1 L system water. Measure temperature so that it falls between 0 ºC and 4 ºC. 4 °C

Add a tea strainer above the ice layer, where individual animals will be placed. The use of the strainer allows cooling the water while avoiding direct contact of the fish body with the ice.

3.2 Transfer animals individually to the strainer. Maintain animals for at least 5 min in contact with the cold water. If animals show any sign of activity, allow for more time in the ice.

4 Decapitate and dissect the animal.

4.1 Transfer the animal to a Petri dish filled with dissection fluid.

Equipment

Cell Culture/Petri Dishes or equivalent	NAME
10X35 mm	TYPE
Nunc™	BRAND
150318	SKU
https://www.thermofisher.com/order/catalog/product/150318#/150318	LINK

4.2 Decapitate the animal by cutting cleanly through the pectoral girdle with dissection scissors. The cut
should be made immediately anterior to the articulation of the pectoral fin with the girdle, severing the heart.

Equipment

Fisherbrand™ Dissecting Scissors	NAME
Scissors	TYPE
Fisherbrand	BRAND
15277168	SKU
https://www.fishersci.co.uk/gb/en/home.html	LINK

4.3 Using the dissecting scissors, remove the skin and bones from the head, exposing the brain. To avoid damaging the forebrain, start dissection at the level of the junction between medulla and spinal cord. Gently raise the medulla with an insulin needle and cut the ventral roots of the cranial nerves using microdissection pincers.

4.4 The tissue sample can be assessed as a whole, or microdissected into forebrain, midbrain, and hindbrain.

ECF extraction

5 In a 1.5 mL microtube filled with extraction fluid, add one brain (or fraction). Keep the microtube on ice or on the fridge, maintaining the temperature at 4 ºC. Incubate for 30 min. 30m

4 °C

00:30:00

Quantification

6 Samples can be analyzed using sensitive techniques, such as HPLC, to assay neurotransmitter content.

Expected result

Since the tissue is not used during the analysis, most of the neurotransmitter content is expected to represent extracellular levels (i.e., released or not transported.

6.1 The remaining tissue can be used to assay other analytes, such as second messenger levels.

Protocol references

Pradel G, Schachner M, Schmidt R (1999). Inhibition of memory consolidation by antibodies against cell adhesion molecules after active avoidance conditioning in zebrafish. *Journal of Neurobiology* 39: 197-206. https://doi.org/10.1002/(SICI)1097-4695(199905)39:2%3C197::AID-NEU4%3E3.0.CO;2-9

Maximino C, Puty B, Benzecry R, Araújo J, Lima MG, Batista EJO, Oliveira KRM, Crespo-Lopez ME, Herculano AM (2013). Role of serotonin in zebrafish (*Danio rerio*) anxiety: Relationship with serotonin levels and effect of buspirone, WAY 100635, SB 224289, fluoxetine and *para*-chlorophenylalanine (pCPA) in two behavioral models. *Neuropharmacology* 71: 83-97. https://doi.org/10.1016/j.neuropharm.2013.03.006

Citations

Step 1

Maximino C, Puty B, Benzecry R, Araújo J, Lima MG, de Jesus Oliveira Batista E, Renata de Matos Oliveira K, Crespo-Lopez ME, Herculano AM. Role of serotonin in zebrafish (Danio rerio) anxiety: relationship with serotonin levels and effect of buspirone, WAY 100635, SB 224289, fluoxetine and para-chlorophenylalanine (pCPA) in two behavioral models.
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Step 2

Vargas R, Jóhannesdóttir IT, Sigurgeirsson B, Thorsteinsson H, Karlsson KA. The zebrafish brain in research and teaching: a simple in vivo and in vitro model for the study of spontaneous neural activity.
https://doi.org/10.1152/advan.00099.2010

Step 3

Wallace CK, Bright LA, Marx JO, Andersen RP, Mullins MC, Carty AJ. Effectiveness of Rapid Cooling as a Method of Euthanasia for Young Zebrafish (Danio rerio).
https://www.ingentaconnect.com/content/aalas/jaalas/2018/00000057/00000001/art00009