

Materials List for:

Preparation of a High-quality Primary Cell Culture from Fish Pituitaries

Eirill Ager-Wick¹, Kjetil Hodne¹, Romain Fontaine¹, Kristine von Krogh¹, Trude M. Haug², Finn-Arne Weltzien¹

¹Department of Basic Sciences and Aquatic Medicine, Faculty of Veterinary Medicine, Norwegian University of Life Sciences

²Department of Oral Biology, Faculty of Dentistry, University of Oslo

Correspondence to: Finn-Arne Weltzien at finn-arne.weltzien@nmbu.no

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Materials

Name	Company	Catalog Number	Comments
Dulbecco's Phosphate Buffered Saline (dPBS), without calcium chloride and magnesium chloride	Sigma (Merck, Damstadt, Germany)	D8537	Adjust solution to pH 7.75 with 1M NaOH and 290 mOsm with mannitol.
L-15 medium (Leibovitz), without L-glutamine	Sigma (Merck, Damstadt, Germany)	L5520	Supplement 500 ml culture medium with 10 mM NaHCO ₃ , 4.5 mM glucose, 2 mM Glutamax. Adjust solution to 290 mOsm with mannitol and filter solution through a 0.2 µm PES sterile filter, before adding 2.5 mL Penicillin-Streptomycin solution (see below for details).
NaOH	Sigma (Merck, Damstadt, Germany)	S5881	Add drops of 1 M solution to increase pH of dPBS and culture medium to 7.75.
NaHCO ₃	Sigma (Merck, Damstadt, Germany)	S5761	10 mM NaHCO ₃ equals 420 mg per 500 mL culture medium.
D-mannitol	Sigma (Merck, Damstadt, Germany)	63565	Use to increase osmolality of dPBS and culture medium. Calculate correct amount needed to reach an osmolality of 290 mOsm.
D-glucose	Sigma (Merck, Damstadt, Germany)	G5400	4.5 mM D-glucose equals 405 mg per 500 mL culture medium.
GlutaMAX Supplement	Gibco (Life Technologies, Paisley, UK)	35050-061	Alternative to L-glutamine, with increased stability. 2 mM Glutamax equals 5 ml of 100x stock in 500 mL culture medium.
Penicillin-Streptomycin	Sigma (Merck, Damstadt, Germany)	P0781	Stock solution 10,000 units penicillin and 10 mg streptomycin per mL. Use 2.5 mL of stock solution in 500 mL L-15 medium (equivalent of 50 U/mL Penicillin and 50 µg/mL Streptomycin).
Trypsin type II-S	Sigma (Merck, Damstadt, Germany)	T7409	Prepare 1 mg/mL in dPBS solution.
Trypsin inhibitor type I-S	Sigma (Merck, Damstadt, Germany)	T6522	Prepare 1 mg/mL in dPBS solution, supplement with 2 µg/ml Dnase I (see details below).
Dnase I	Sigma (Merck, Damstadt, Germany)	D5025	Use in trypsin inhibitor solution (see above).
0.2 µm Polyethersulfone (PES) sterile filter system	Corning Inc. (Corning, NY)	431097	Use for sterile filtration of dPBS and L-15 medium after adjustments.

35 mm cell culture dish with glass bottom, poly d-lysine coated	MatTek Corporation (Ashland, MA)	P35GC-1.5-10-C	Can also be replaced by plastic dish, depending on downstream application.
Dumont #5 fine forceps	Fine Science Tools (CA)	11254-20	Straight tip
Dumont #5/45 fine forceps	Fine Science Tools (CA)	11253-25	Angled 45° tip
Stereo microscope SZ61	Olympus Corp. (Tokyo, Japan)		Use for dissection of pituitaries.
Alegre X-22R Centrifuge	Beckman Coulter Inc. (Brea, CA)		With cooling option (similar to current model XR-30).
Water bath	Techne (Staffordshire, UK)		Any water bath with the possibility of adjusting temperature will do.
Pasteur glass pipettes	VWR (NY)	612-1701	Outer diameter 1.6 mm, fire polish and autoclave before use.
Galaxy MiniStar table centrifuge	VWR (NY)	521-2844	Any small table centrifuge will do.
Fine needles/insect pins	Fine Science Tools (CA)	26001-40	Diameter 0.03 mm. Other fine needles can be used instead.
Wax plate			Custom made by adding melted paraffin wax in large petri dish.