

Title	DME/F12+ITS medium preparation			
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Approval section:

Position	Test facility manager:	Study director	QR:
Name			
Signature			
Location, date			

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1 PURPOSE

Instructions for DME/F12+ITS cell culture medium preparation

- Buffered version for use in 5% CO₂ incubator
- Unbuffered for use with IMOLA

2 SCOPE

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3 EQUIPMENT AND MATERIALS

3.1 TOOLS

Pos	Tool
1	Refrigerator
2	Balance
3	Magnetic stirrer
4	Aspirator
5	1L volumetric flask
6	Serological pipettes
7	Bottle-top vacuum filters 0.2 µm
8	pH-Meter
9	Osmometer
10	0.5 L – sterile bottles
11	Digital, waterproof temperature sensor
12	Laminar flow hood

3.2 REAGENTS

Pos	Reagent
1	Double-distilled water (ddH ₂ O)
2	DMEM (Dulbecco's Modified Eagle's Medium) with 4500 mg/L glucose and L-glutamine, without sodium bicarbonate, powder, suitable for cell culture (e.g., Sigma D5648)
3	Nutrient Mixture F-12 Ham with L-glutamine, without sodium bicarbonate, powder, suitable for cell culture (e.g., Sigma N6760)
4	ITS Liquid Media Supplement (100x) liquid, sterile-filtered, BioReagent, suitable for cell culture (e.g., Sigma I3146)
5	Sodium chloride – NaCl BioReagent, suitable for cell culture (e.g., Sigma S5886)
6	Sodium bicarbonate - NaCHO ₃ powder, BioReagent, for molecular biology, suitable for cell culture (e.g., Sigma 5761)
7	HCl 0.1M, 0.5M
8	NaOH 0.1M, 0.5M

3.3 SAFETY REQUIREMENTS

	• Put on gloves and a lab coat • Use 70% ethanol for the item's disinfection • Vacuum filtration is to be performed under the laminar flow hood
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3.4 STORAGE REQUIREMENTS

Storage at 6°C required.

4 PREPARATION OF MEDIUM

Pos	Instruction	Picture/Notes
1	Document medium preparation in the reagents logbook • LOT# of the cell-culture medium • Short name • Reagents' information (LOT#, manufacturer, type)	LOT# syntax: RYYMMDD-XX e.g. R170522-01 e.g. JWI e.g. Sigma D5648, #563924, DMEM high-glucose
2	Use 70% ethanol disinfectant for: • Laminar flow hood • Hands • Aspirator hose • Exterior sides of the storage bottles	
3	Preparation of 500 mL DMEM medium: • Position 1 L volumetric flask on magnetic stirrer and add 0.5 L (+/- 0,5%) ddH₂O • Weigh 6.7 g (+/- 0,5%) of DMEM, put it into a volumetric flask, and stir • Label open DMEM vial and put it back into the fridge For unbuffered medium: • Add 1.52 g (+/- 0,5%) NaCl For buffered medium: • Add 0.6 g (+/- 0,5%) NaCl and 0.92 g (+/- 0,5%) NaCHO₃ • Do not adjust pH	Prepare either buffered (e.g., for feeding of cells in 5% CO ₂ incubator) or unbuffered (e.g., for IMOLA experiments) medium Document process in Reagent's logbook For example: Unbuffered DMEM medium: 0.5 L ddH ₂ O + 6.7 g DMEM (D5648, #563924) + 1.52 g NaCl (Sigma S5886, #54354)
4	Preparation of 500 mL F12 medium: • Position 1 L volumetric flask on magnetic stirrer and add 0.5 L (+/- 0,5%) ddH₂O • Weigh 5.3 g (+/- 0,5%) of F12-Ham, put it into volumetric flask and stir • Label open F12-Ham vial and put it back into the fridge	Document process in the Reagents logbook For example: Unbuffered F12-Ham medium: 0.5 L ddH ₂ O + 5.28 g DMEM (Sigma N6760, #73625) + 1.22 g NaCl (Sigma S5886, #54354)

Pos	Instruction	Picture/Notes
	For unbuffered medium: - Add 1.22 g (+/- 0,5%) NaCl For buffered medium: - Add 0.3 g (+/- 0,5%) NaCl and 0.92 g (+/- 0,5%) NaCHO₃	
5	Preparation of 1 L DMEM/F12+ITS - Mix: - 475 mL (+/- 0,5%) DMEM - 475 mL (+/- 0,5%) F-12 Ham - 5 mL (+/- 0,5%) ITS (100x) - 45 mL (+/- 0,5%) ddH₂O For unbuffered medium: - If any additional substances (e.g., FBS, Gentamicin) are added to the medium, add them before pH adjustment - Adjust to pH 7.4 +/- 1% with NaOH and HCl; monitor pH changes at least for 30 min after adding NaOH or HCl for the last time - Adjust to 340 mOsmol/kg +/- 1% using ddH₂O or NaCl - If osmolarity has been adjusted, pH has to be checked and, if required, adjusted again For buffered medium: - Do not adjust pH - Adjust to 340 mOsmol/kg +/- 1% using ddH₂O or NaCl	If any reagent has been stored in the fridge before the pH adjustment, leave the medium at room temperature for at least 3h. Check if the medium has reached room temperature using a digital temperature sensor Handle the gentamicin stock solution in laminar flow Final Gentamicin concentration in medium: 50 µg/ml FBS concentration in volume/volume For example: 5% (v/v) in 100 mL 95 mL DMEM and 5 mL FBS Document process in the Reagents logbook For example: pH 7.38 Osmolarity: 338 mOsmol/kg
6	Sterile filter into 2x 500 mL bottles	Work in laminar flow
7	Label medium flasks: - Name of the medium - LOT# of the medium - Date of expiration - Your short name	Date of expiration = date of preparation + 6 months
8	Store at 6 °C	
End		

5 ARCHIVAL STORAGE

The original paper version of this document in its approved and released status is stored in the cellasys laboratory's archive.

6 RESPONSIBILITIES

Creation	Study Director
Approval	Testing Facility Manager
Archivation	Archivation Manager
Processing	Testing Personnel

7 REFERENCES

Manuals of the equipment listed in the tools section of this document. The manuals can be found in the Manuals folder, located in the laboratory.

8 ANNEXES

None.