

<b>Title</b>	<b>DME/F12+ITS medium preparation</b>			
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<b>Abstract</b>	Procedure to prepare cell culture medium DME/F12+ITS (buffered or unbuffered)			
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**Approval section:**

Position	<b>Test facility manager:</b>	<b>Study director</b>	<b>QR:</b>
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Signature			
Location, date	Kronburg,		

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## 1 PURPOSE

Instructions for DME/F12+ITS cell culture medium preparation

- Buffered version for use in 5% CO<sub>2</sub> incubator
- Unbuffered for use with IMOLA-IVD

## 2 SCOPE

cellasys Laboratories, S1/S2

## 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 <sub>2</sub> 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 <sub>3</sub> 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

	<ul style="list-style-type: none"> <li>• Put on gloves and a lab coat</li> <li>• Use 70% ethanol for the item's disinfection</li> <li>• Vacuum filtration is to perform under the laminar flow hood</li> </ul>
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### 3.4 STORAGE REQUIREMENTS

Storage at 6°C required.

## 4 PREPARATION OF MEDIUM

Pos	Instruction	Picture/Notes
1	<p><b>Document medium preparation in reagents logbook</b></p> <ul style="list-style-type: none"> <li>• LOT# of the cell-culture medium</li> <li>• Short name</li> <li>• Reagents' information (LOT#, manufacturer, type)</li> </ul>	<p>LOT# syntax: RYYMMDD-XX e.g. R170522-01</p> <p>e.g. JWI</p> <p>e.g. Sigma D5648, #563924, DMEM high-glucose</p>
2	<p><b>With 70% ethanol alcohol disinfect:</b></p> <ul style="list-style-type: none"> <li>• Laminar flow hood</li> <li>• Hands</li> <li>• Aspirator hose</li> <li>• Exterior sides of the storage bottles</li> </ul>	
3	<p><b>Preparation of 500 mL DMEM medium:</b></p> <ul style="list-style-type: none"> <li>• Position 1 L volumetric flask on magnetic stirrer and add 0.5 L (+/- 0,5%) ddH<sub>2</sub>O</li> <li>• Weigh 6.7 g (+/- 0,5%) of DMEM, put it into volumetric flask and stir</li> <li>• Label open DMEM vial and put it back into the fridge</li> </ul> <p><b>For unbuffered medium:</b></p> <ul style="list-style-type: none"> <li>• Add 1.52 g (+/- 0,5%) NaCl</li> </ul> <p><b>For buffered medium:</b></p> <ul style="list-style-type: none"> <li>• Add 0.6 g (+/- 0,5%) NaCl and 0.92 g (+/- 0,5%) NaCHO<sub>3</sub></li> <li>• Do not adjust pH</li> </ul>	<p>Prepare either <b>buffered</b> (e.g. for feeding of cells in 5% CO<sub>2</sub> incubator) or <b>unbuffered</b> (e.g. for IMOLA experiments) medium</p> <p>Document process in Reagent's logbook</p> <p>For example:</p> <p>Unbuffered DMEM medium: 0.5 L ddH<sub>2</sub>O + 6.7 g DMEM (D5648, #563924) + 1.52 g NaCl (Sigma S5886, #54354)</p>
4	<p><b>Preparation of 500 mL F12 medium:</b></p> <ul style="list-style-type: none"> <li>• Position 1 L volumetric flask on magnetic stirrer and add 0.5 L (+/- 0,5%) ddH<sub>2</sub>O</li> <li>• Weigh 5.3 g (+/- 0,5%) of F12-Ham, put it into volumetric flask and stir</li> <li>• Label open F12-Ham vial and put it back into the fridge</li> </ul> <p><b>For unbuffered medium:</b></p>	<p>Document process in Reagents logbook</p> <p>For example:</p> <p>Unbuffered F12-Ham medium: 0.5 L ddH<sub>2</sub>O + 5.28 g DMEM (Sigma N6760, #73625) + 1.22 g NaCl (Sigma S5886, #54354)</p>

Pos	Instruction	Picture/Notes
	<ul style="list-style-type: none"> <li>Add 1.22 g (+/- 0,5%) NaCl</li> </ul> <p><b>For buffered medium:</b></p> <ul style="list-style-type: none"> <li>Add 0.3 g (+/- 0,5%) NaCl and 0.92 g (+/- 0,5%) NaCHO<sub>3</sub></li> </ul>	
5	<p><b>Preparation of 1 L DMEM/F12+ITS</b></p> <ul style="list-style-type: none"> <li>Mix: <ul style="list-style-type: none"> <li>- 475 mL (+/- 0,5%) DMEM</li> <li>- 475 mL (+/- 0,5%) F-12 Ham</li> <li>- 5 mL (+/- 0,5%) ITS (100x)</li> <li>- 45 mL (+/- 0,5%) ddH<sub>2</sub>O</li> </ul> </li> </ul> <p><b>For unbuffered medium:</b></p> <ul style="list-style-type: none"> <li>If any additional substances (e.g. FBS, Gentamicin) are added to the medium, add them before pH adjustment</li> <li>Adjust to <b>pH 7.4 +/- 1%</b> with NaOH and HCl; monitor pH changes at least for 30 min after adding NaOH or HCl for the last time</li> <li>Adjust to <b>340 mOsmol/kg +/- 1%</b> using ddH<sub>2</sub>O or NaCl</li> <li>If osmolarity has been adjusted, pH has to be checked and, if required, adjusted again</li> </ul> <p><b>For buffered medium:</b></p> <ul style="list-style-type: none"> <li>Do not adjust pH</li> <li>Adjust to <b>340 mOsmol/kg +/- 1%</b> using ddH<sub>2</sub>O or NaCl</li> </ul>	<p>If any reagent has been stored in the fridge before the pH adjustment, <b>leave the medium at room temperature for at least 3h</b>. Check if medium has reached room temperature using a digital temperature sensor</p> <p>Handle Gentamicin stock solution in laminar flow Final Gentamicin concentration in medium: <b>50 µg/ml</b></p> <p>FBS concentration in volume/volume For example: 5% (v/v) in 100 mL 95 mL DMEM and 5 mL FBS</p> <p>Document process in Reagents logbook</p> <p>For example: pH 7.38 Osmolarity: 338 mOsmol/kg</p>
6	<b>Sterile filter into 2x 500 mL bottles</b>	Work in laminar flow
7	<p><b>Label medium flasks:</b></p> <ul style="list-style-type: none"> <li>Name of the medium</li> <li>LOT# of the medium</li> <li>Date of expiration</li> <li>Your short name</li> </ul>	Date of expiration = date of preparation + 6 months
8	<b>Store at 6 °C</b>	
<b>End</b>		

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

## 8 ANNEXES

None.