

## **Protocol**

# Production of **CHOlean Medium** from powder

Please note, this document may be periodically updated in order to ensure the most current practices are in place. It is the user's responsibility to ensure the lastest release of this protocol is applied. Valid versions are made available via Xell's webshop.

### Production of liquid medium with the CHOlean Medium Powder Kit

#### Material:

- We recommend preparing the whole Powder Kit in a single batch! For that, please adjust the amounts/volumes per L given in this protocol according to your container/batch size!
- CHOlean Medium Powder (22.26 g/L) Xell Cat. 1140-XXXXDPM
- approx. 1 L H<sub>2</sub>O per L medium (WFI or equivalent quality)
- 1.80 g/L NaHCO<sub>3</sub> Ph. Eur.
- We recommend wearing a dust mask during preparation!



**Visual control:** 

Container

A.

Sealed and without any damage.

B. Appearance **Free flowing powder** (record color).



Procedure: Check:

1.	15 - 35 °C	Fill <b>0.8 L</b> per 1 L final medium <b>15-35 °C water (WFI or equivalent quality)</b> into the stirred tank/blending vessel.  Adjust volume according to batch size.	
2.		Start the stirrer of the system. Due to foam formation during medium production, the vortex should not reach the stirrer.	
3.		Add <b>22.26 g/L of CHOlean Medium powder</b> slowly to the stirred water. Avoid clumping.	
		Adjust volume according to batch size.  Note: We recommend preparing the whole powder kit at once.	

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4.	H <sub>2</sub> O	Rinse the empty medium container with a suitable amount of <b>water</b> (WFI or equivalent quality) and pour liquid into the stirred tank.	
5.	30 min	Stir for <b>30 minutes.</b> Note: The powder will not completely dissolve at this stage at a pH range of 7.0 - 7.5.	
6.	NaOH	Titrate with <b>8 M NaOH to pH 8.5 - 8.7</b> (usually between 1.0 to 1.4 mL/L of 8 M NaOH is required) and adjust volume to batch size. <b>Note:</b> The powder should be completely dissolved, but the solution should be remain turbid.	$\bigcirc$
7.	Stir for further 30 minutes.  Note: The powder will not completely dissolve at this stage.		$\bigcirc$
8.	Titrate with 6 M HCl to pH 6.0 - 6.5 (usually between 1.5 to 2.5 of 6 M HCl is required) and adjust volume to batch size.  Note: The powder should be completely dissolved and the solution should be clear.		$\bigcirc$
9.	30 min	Stir for further 30 minutes.  Note: The powder will not completely dissolve at this stage.	
10.	Add <b>1.80 g/L NaHCO</b> <sub>3</sub> to the stirred tank.  Adjust volume according to batch size.		
11.	10 min	Stir for <b>5 - 10 minutes</b> . <b>Note:</b> The powder will be completely dissolved at this stage at a pH range of 6.8 - 7.5.	

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12.	NaOH or HCl	If needed titrate with <b>8 M NaOH or 6 M HCl to pH 7.1 - 7.6. Note:</b> The powder should be completely dissolved and the solution should be clear.	
13.	100 %	Add an appropriate volume of <b>water</b> (WFI or equivalent quality) to reach the final volume.  Final volume depends on batch/container size!	
14.	10 min	Stir for <b>5-10 minutes</b> .	
15.	Check pH (pH 7.1 - pH 7.6) and osmolality (300 mOsmol/kg ± 15 mOsmol/kg).		0
16.		The Medium can now be <b>sterile filtered</b> (0.45 µm + 0.1µm) and <b>bottled</b> .	$\bigcirc$

### **Change History:**

Revision	Date	Author	Comment/Description
01-02	n/a	n/a	Initial version
03	03.12.2021	SST	Addition of change history
04	18.03.2022	AWU	Changed procedure, due to formulation correction

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