



Customized solutions for cell cultures.

# Protocol

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## Production of **liquid medium** from HEK ViP NB 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 latest release of this protocol is applied. Valid versions are made available via Xell's webshop.*

## Production of liquid medium from HEK ViP NB powder

### Guideline:

We recommend preparing the whole powder container in a single batch. For that, please adjust the amounts/volumes per liter given in this protocol according to your batch size.

### Material:

- HEK ViP NB powder (use at 19.64 g/L); Xell Cat. 891-XXXXDPM
- H<sub>2</sub>O (WFI or equivalent quality)
- 2.10 g/L NaHCO<sub>3</sub> Ph. Eur.
- 0.4 – 0.8 mL/L 6M Hydrochloric acid (HCl)



### Optional requirement:

- 1 - 2 mL/L Growth hormone supplement (recommended 1.6 ml/L; e.g. Xell Cat. No. 1005-XXXX); alternatively: 0.05 – 0.1 mL/L LONG® R3 IGF-I (Cat. No. 1006-XXXX; recommended 0.08 mL/L)  
*The values are corresponding to the use of 5 - 10 mg/L rInsulin (recommended 8 mg/L); alternatively: 0.05 – 0.10 mg/L LONG® R3 IGF-I (recommended 0.08 mg/L)*

We recommend wearing a dust mask during preparation.

### Visual control:

- A. Container      **Sealed and without any damage.**
- B. Appearance      **Free flowing powder** (record color).

### Check:










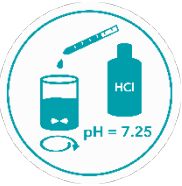



Color: \_\_\_\_\_

### Procedure:

### Check:

1.	15 - 35 °C 80 %	Fill <b>0.8 L</b> per 1 L (80% v/v) final medium solution <b>15-35°C water (WFI or equivalent quality)</b> into the stirred tank/blending vessel.  <b>Note:</b> <i>Deviating temperature may alter dissolution rate. An adaption of time for solubilization might be necessary.</i>	<input type="checkbox"/>
2.	80 %	Start the stirrer of the system. Due to foam formation during medium production, the vortex should not reach the stirrer.	<input type="checkbox"/>

3.		<p>Add <b>19.64 g/L</b> of HEK ViP NB powder slowly to the stirred water. Avoid clumping.</p> <p><b>Note:</b> We recommend preparing the whole powder container at once.</p>	<input type="radio"/>
4.		<p>Rinse the emptied powder container with <b>0.05 L</b> per 1 L final medium solution (5% v/v) of water (WFI or equivalent quality) and pour liquid into the stirred tank.</p>	<input type="radio"/>
5.		<p>Stir for <b>30 minutes</b> with lid closed.</p> <p><b>Note:</b> The powder should be completely dissolved, and the solution should be clear.</p>	<input type="radio"/>
6.		<p>Add <b>2.1 g/L</b> NaHCO<sub>3</sub> Ph. Eur. to the stirred tank.</p>	<input type="radio"/>
7.		<p>Stir for <b>5-10 minutes</b> with lid closed.</p> <p><b>Note:</b> The solution should be clear, without precipitates. If not, stepwise increase mixing time by up to further 10 min.</p>	<input type="radio"/>
8.		<p>Add an appropriate volume of water (WFI or equivalent quality) to reach the final volume.</p> <p><b>Note:</b> Final volume depends on batch/container size.</p>	<input type="radio"/>
9.		<p>Stir for <b>5-10 minutes</b> with lid closed.</p> <p><b>Note:</b> The solution should be clear, without precipitates. If not, stepwise increase mixing time by up to further 10 min.</p>	<input type="radio"/>

10.		<p>Titrate with <b>6 M HCl to pH 7.25 ± 0.25</b> (usually between 0.4 to 0.8 mL/L of 6M HCl is required) and adjust volume to batch size.</p> <p><b>Note:</b> <i>The powder should be completely dissolved, and the solution should be clear.</i></p>	○
11.		<p>Measure pH (<b>7.0 – 7.5</b>) and osmolality (<b>290 ± 15 mOsmol/kg</b>) of the medium.</p>	○
12.		<p><b>Optional requirement:</b>  Add <b>1 - 2 mL/L Growth hormone supplement</b> (recommended 1.6 ml/L; alternatively: 0.05 – 0.1 mL/L LONG® R3 IGF-I (recommended 0.08 mL/L) from Xell stock solutions.</p> <p>The values are corresponding to the use of <b>5 to 10 mg/L rInsulin</b> (recommended 8 mg/L) alternatively: 0.05 – 0.1 mg/L LONG® R3 IGF-I (recommended 0.08 mg/L) directly from powder or an appropriate stock solution.</p> <p><b>Note:</b> <i>Adjustment of growth hormone concentration for optimization is possible but depends on used cell line and application.</i></p>	○
13.		<p>The medium can now be sterile filtered (0.45 µm + 0.2 µm or 0.45 µm + 0.1 µm) and bottled.</p>	○

**Change History:**

Revision	Date	Author	Comment/Description
01	n/a	n/a	Initial version
02	03.12.2021	SST	Addition of change history, adjustment of document steering acc. to amendment 13.04.2021
03	04.10.2024		Change of contact details & company name

For further information or assistance contact us.

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