

# Product Information



## Instructions for Dissolving AppliChem Powder Media to Prepare Sterile Liquid Cell Culture Media

Use bi-distilled or deionized, pyrogen-free water to reconstitute powder media. Powdered media and salts are very hygroscopic and must be stored under dry conditions. After opening one package the whole content must be dissolved at once.

Reconstitute the powder form of media to produce 1X liquid medium, as the different amino acids may precipitate because of their low solubility. They are able to form salts which are of low solubility in concentrated solutions.

If supplements are needed, they may be added before filtration (unsterile) or after filtration (sterile).

### Procedure 1: Preparing *sterile filtered* liquid media

- i. Add deionized, bi-distilled pyrogen-free water to the required quantity of powdered medium (use approx. 90 % of the required amount of water so as to adjust the pH later). Flush out any remaining powder from the container. Stir until completely dissolved. The temperature of the water should be between +15 and +30 °C
- ii. When the powder is completely dissolved, add sodium hydrogen carbonate and dissolve completely as well. The required quantities are listed in the table in our General Catalog. (This table is also available from the AppliChem website).
- iii. Adjust the pH (physiological optimum is pH 6.8 - 7.2) with 1 N HCl or 1 N NaOH while stirring.

**NOTE:** The pH should be approx. 0.2 units lower than the target pH, because the pH will slightly rise during filtration when the CO<sub>2</sub> leaks out.

- iv. After adjusting the pH add water up to the appropriate final volume and mix it very well and sterile filter immediately.
- v. Store the medium at +4 °C protected from light.

### Procedure 2: Preparing liquid media from *autoclavable* powder media

- i. Add deionized, bi-distilled pyrogen-free water to the required quantity of powdered medium (use approx. 90 % of the required amount of water so as to adjust the pH later). Flush out any remaining powder from the container. Stir until completely dissolved. The temperature of the water should be between +15 and +30 °C
- ii. Adjust the pH (physiological optimum is pH 6.8 - 7.2) with 1 N HCl or 1 N NaOH while stirring. **NOTE:** The pH should be approx. 0.2 units lower than the target pH, because the pH will slightly rise during autoclaving when the CO<sub>2</sub> leaks out.
- iii. After adjusting the pH add water up to the appropriate volume (e.g. 970 ml) and mix well.
- iv. Autoclave.
- v. After autoclaving add a sterile-filtered solution of 7.5 % sodium hydrogen carbonate according to the table in our General Catalog\* and dissolve completely as well (e.g. 30 ml of 7.5 % NaHCO<sub>2</sub>-solution/ L).  
\* This table is also available from the AppliChem website.
- vi. Store the medium at +4 °C protected from light.

# Product Information



## Procedure 3: Preparing liquid *insect cell culture media*

- i. Add deionized, bi-distilled pyrogen-free water to the required quantity of powdered medium (use approx. 90 % of the required amount of water so as to adjust the pH later). Flush out any remaining powder from the container. Stir until completely dissolved. The temperature of the water should be between +15 and +30° C
- ii. When the powder is completely dissolved, add sodium hydrogen carbonate and dissolve completely as well. The required quantities are listed in the table in our General Catalog. (This table is also available from the AppliChem website).
- iii. Adjust to pH **pH 6.3\*** with 1 N HCl or 1 N NaOH while stirring.

**NOTE:** The pH should be approx. 0.2 units lower than the target pH, because the pH will slightly rise during filtration when the CO<sub>2</sub> leaks out.

\**Comment:* The physiological pH optimum of insect cell culture media is more acidic compared to the pH of mammalian cell culture media.

At pH values above approx. 6.5 an undesired precipitation will occur with insect cell culture media which contains a high concentration of calcium chloride.

- iv. After adjusting the pH add water up to the desired volume, mix it very well and filter immediately.
- v. Store the medium at +4° C protected from light.

version: MM090707