Technical Info

Well advised with Roth.

Guidelines for the production and storage of media and agars

The preparation of agars and media is a simple and quick process in daily laboratory work. These brief guidelines intend to illustrate some points, which don't involve much work and which will guarantee you the highest quality media and agars.

REHYDRATION (DISSOLVING)

Basically, we do not recommend autoclaving the anhydrous media directly after mixing with water but rather dissolving the powder completely prior to sterilisation. Make sure to mix the powder completely with water. In many cases we also recommend heating, possibly until the solution boils. In fact, this dissolution process before sterilisation generally determines the clarity and yield of the end product and guarantees, particularly with highly concentrated stock solutions (e.g. 10 x LB-medium), an optimal end product. Hereby, a homogeneous solution should be produced with minimal heat stress, and only pure water (distilled or deionised) should be used. The process is described below in general; however, please also follow the instructions for each specific culture medium, which can be found on the label or data sheet.

Add the required amount of anhydrous medium to about half a volume of water (e.g. 500 ml for 1 l preparation) and mix well. Please take care that no lumps are formed. After mixing thoroughly, add water up to the final volume (e.g. 1 l) and mix again. Please be sure to dissolve any residual powder sticking to the glass. Let the mixture stand for five minutes at room temperature; this will help to obtain a uniform suspension. Some compositions which do not contain gelatine, agar or cystine can be dissolved without heating. Following resuspension, these media may be placed directly into the autoclave and be sterilised (see below). Other media must be heated under stirring in order to dissolve thoroughly and to avoid retardation of boiling. Please ensure even heat distribution and boil the preparation for as short a time as possible. When using a preparation of 1 l with 1 x concentrated medium, 1 minute is usually enough. However, we recommend extending the time to 2-3 minutes with more highly concentrated solutions. The media can then be sterilised in the autoclave after a short cooling period (see below).

STERILISATION

Again, please follow the instructions on the labels or data sheets for each specific culture medium. In general, these instructions apply to smaller preparations, e.g. 1 l with 1 x concentrated solution. Sterilisation time for larger amounts should be extended by up to 30 minutes without exceeding the temperature specified for each medium. We also recommend extending the autoclaving time accordingly when using more highly concentrated media (e.g. 10 x stock solutions). Please note: media containing carbohydrates should not be autoclaved at a temperature of over 116 °C to 118 °C. Overheating must always be avoided in such cases. Only apply short autoclaving times of approx. 10 minutes as the heating and cooling phases must also be added. The media should be cooled down to room temperature after autoclaving before being placed in cold storage. Antibiotics are not thermally stable and should also only be added to cooled (approx. 50-55 °C) media. For casting Petri dishes, allow the agar media to cool down to approx 55 °C, add any additional antibiotics or other additives and pour layers of approx 5 mm into the Petri dishes. When adding additives, please follow the instructions on the corresponding data sheet or label.

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STORAGE OF LIQUID MEDIA OR AUTOCLAVED AGAR

Although autoclaved media are sterile, however the components, as natural products, are subject to natural decay. Adequate cooling can slow down the decomposition, but cannot stop it. We therefore recommend storing all autoclaved media for a few weeks only at 4-8 °C. This period may have to be further reduced with certain additives such as defibrillated blood. Media containing antibiotics is basically more stable; however the concentration of antibiotics diminishes during this time. We recommend not storing plates and media for more than 2 weeks.

STORAGE OF DRY MEDIA

Store the powder in its original bottle in a cool and dry place at room temperature, but if possible under 25 °C and without direct sunlight. Please note: some anhydrous media must be stored at a temperature of 2-8 °C (see label and respective data sheet). Once a bottle with anhydrous media has been opened for use, it should be closed immediately to avoid moisture absorption. If the media becomes damp and lumps form, it will be contaminated and sterilisation will be difficult. In this case, the bottle should be discarded. The dry powder of microbiological culture media and agar usually keeps for about 2 years after delivery if adequately and correctly stored.

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