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Application Note

Addition of Supplements to ReadyStream® Media

Introduction

The ReadyStream® system is revolutionizing the preparation of enrichment culture media for microbiological food testing. In the standard ReadyStream® operating procedure the system is used with unsupplemented Tryptic Soy Broth (TSB) or Buffered Peptone Water (BPW). However, using a special technique, it is also possible to conveniently add supplements, required by regulations or according to alternative methods, to the ReadyStream® media. This application note gives an overview of supplements that can be added to TSB or BPW and proposes a laboratory procedure for this to be performed.

Regulatory guidance of supplement addition to BPW and TSB

Table 1 lists some food testing regulatory standards that require supplements to be added to TSB or BPW, along with instructions on how to prepare the required supplement solutions in the lab. Details on the procedure of adding a supplement solution to reconstituted ReadyStream® culture medium are described in the following chapter.

This list may not be exhaustive. Especially supplements described in alternative methods are not included. However, to add other supplements to the listed culture media, an analogous procedure can usually be followed.

Table 1: Regulatory standards requiring supplements to be added to BPW or TSB

Media	Supplement	Standard	Lab instructions	Additional information
TSB	Ferrous sulfate ($FeSO_4$)	FDA-BAM Chapter 5; 2a	Dissolve 35 mg $FeSO_4$ in 10 mL of deionized (DI) water, sterilize by filtration, then add to 1.0 liter of ReadyStream® TSB media	BAM Media M186 for testing of liquid whole eggs (homogenized) Trypticase (Tryptic) Soy Broth with ferrous sulfate: Trypticase peptone 17 g; Phytone peptone 3 g; NaCl 5 g; K_2HPO_4 2.5 g; Glucose 2.5 g; Ferrous sulfate 35 mg; Distilled water 1.0 liter
TSB	K_2SO_3	FDA-BAM Chapter 5; 10b	Dissolve 5 g K_2SO_3 in 0.1 L of TSB, sterilize by filtration, then mix into 0.9 L ReadyStream® TSB media (see further instructions below)	For testing of onion flakes, onion powder, garlic flakes
TSB	Yeast extract	FDA-BAM Chapter 6	Dissolve 6 g Yeast Extract in 0.1 L of TSB, sterilize by filtration, then mix into 0.9 L ReadyStream® TSB media (see further instructions below)	BAM Media M157 for enrichment of Shigella Trypticase Soy Broth with 0.6% Yeast Extract (TSBYE): Trypticase soy broth 30 g; Yeast extract 6 g; Distilled water 1 liter. Note that adding novobiocin to the enrichment medium may be incompatible with detection by DNA hybridization.
TSB	Yeast extract	ISO / TS 18867:2015	Dissolve 6 g Yeast Extract in 0.1 L TSB, sterilize by filtration, then mix into 0.9 L ReadyStream® TSB media (see further instructions below)	Detection of pathogenic *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* Culture medium to enrich *Yersinia pseudotuberculosis* for subsequent detection by PCR
TSB	Polymyxin B single-strength	ISO 21871: 2006	Dissolve 111111 IU of Polymyxin B in 10 mL DI water, sterilize by filtration, then add to 1.0 L ReadyStream® TSB media	Horizontal method for the determination of low numbers of presumptive *Bacillus cereus*
BPW	Crystal Violet	MLG / USDA 4.14	Per 2.925 L of BPW add 1 mL of 1% aqueous solution of crystal violet.	For fermented products

Proposed laboratory procedure

The suggested best-practice procedure for preparing supplemented ReadyStream® media involves five steps (see below). Depending on individual lab conditions alternative practices might be more suitable and efficient.

1. **Aseptic techniques:** Ensure that all procedures are carried out using aseptic techniques to prevent contamination. Ideally, perform the following steps in a laminar flow hood or under sterile conditions.
2. **Prepare the supplement solution:** Prepare the supplement solution separately according to the supplier's instructions. If the supplement is a solid, it must be dissolved, depending on its solubility in either deionized (DI) water or in the culture media to which it is to be added. DI water can be used as solvent if all the supplement can be dissolved in ≤1% of the media volume to which it will be added, e.g. in 10 mL of DI water if the solution is then added to at least 1000 mL of media. If the required solvent volume is >1% of the media volume to which it is to be added, the same media should be used as solvent to avoid unacceptable dilution of the media. Give the required amounts of supplement and solvent into a suitable vessel and homogenize the solution.
3. **Set up bottle and connectors:** Follow the instructions outlined in the **ReadyStream® Dilutor Connector Accessories – User Guide** to set up the connectors and the GL45 lab bottle into which supplement solution from the vessel (step 2) and media from the ReadyStream® system are going to be filled. Choose the setup described in the guide that suits your lab workflow and practices best. Note that the GL45 bottle must be large enough for the volume of culture media to be dispensed and the supplement solution to be added (see **Table 1** for appropriate ratios).
4. **Add the supplement solution:** To add the supplement solution to the GL45 bottle, first take off the gray ReadyStream® connector from the tube connector (**Cat no. RDYFCON01/RDYFCON05**). Then inject the required volume of the supplement solution with a syringe and a connected syringe sterile filter (for example **Cat no. SLGVM33RS**) through the tube connector into the bottle. The volume of the supplement solution to inject depends on the required concentration of supplement in the media as stipulated in the regulation (see **Table 1**) and the total volume of media added to the GL45 bottle (see step 5 below).
5. **Fill media from ReadyStream® system into bottle:** For filling of the bottle with media with the **ReadyStream® system, proceed as described in the ReadyStream® Dilutor Connector Accessories – User Guide**.
   If the supplement has been dissolved in the respective media, e.g. 100 mL (see point 2), this volume needs to be subtracted from the final media volume, to obtain the media volume that needs to be dispensed by the ReadyStream® system, adding up to the final media volume. If not the full nominal bottle volume is filled, for example, if there is remaining media left in the bottle, adjust the amount of supplement according to the media volume added.
   Make sure the media and supplement are well homogenized for example by using a magnetic stir bar. For further processing of the supplemented media, e.g. dispensing through a dilutor, please see **ReadyStream® Dilutor Connector Accessories – User Guide**

For further information on the ReadyStream® system, Scan the QR code.

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