Bacto™ CD Supreme Fermentation Production Medium (FPM)

Catalog Number A4973701 and A4973702

Pub. No. MAN0024960 Rev. A.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

Bacto™ CD Supreme Fermentation Production Medium (FPM) is a chemically defined microbial medium designed for plasmid DNA or recombinant protein production.

Bacto™ CD Supreme Fermentation Production Medium (FPM) is designed specifically for expression systems utilizing *Escherichia coli* and is recommended for use in microbial cell cultures, as a base medium, and to enhance overall culture performance in batch or fed-batch processes. This medium requires the addition of a carbon source, glycerol or glucose are recommended, but may vary depending on process requirements.

Contents and storage

Contents	Cat. No.	Amount	Storage	Shelf Life
Bacto™ CD Supreme Fermentation Production Medium (FPM)	A4973701	500 g	Store at 2°C to 8°C.	18 months
	A4973702	10 kg		

Required materials not supplied

Unless otherwise indicated, all materials are available through **www.thermofisher.com**.

Product	Cat. No.
Glucose	15023021
Glycerol	17904
Ampicillin sodium salt	11593027
IPTG (isopropyl-beta-D-thiogalactopyranoside)	34060
Escherichia coli cells (examples include: DH5a and BL21)	Thermo Fisher Scientific™
Luria-Bertani (LB) Agar with Ampicillin (100 µg/mL) plates – 12 mm × 85 mm Monoplate LB Agar with Ampicillin (100 µg/mL) plates – 15 mm × 150 mm Monoplate	R110204 R110846
Nalgene™ 0.2 µm Rapid Flow Filters	122-0020
GeneJET™ Plasmid Miniprep Kit	K0502 (50 preps) or K0503 (250 preps)
Gibco™ L-Proline, USP/EP/JP	A37802IN
Gibco™ L-Leucine, USP/EP/JP	A37732IN
Gibco™ L-Isoleucine, USP/EP/JP	A37731IN

For Research Use or Further Manufacturing. Not for diagnostic use or direct administration in humans or animals.

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Procedural guidelines

- Bacto™ CD Supreme Fermentation Production Medium (FPM) is hygroscopic and should be stored at 2–8°C in a dry place away from sunlight and extreme temperature variation.
- Once the powder bottle has been opened for initial use, it should be tightly closed as soon as possible to protect the powder from absorbing moisture.
- Do not use if the bottle or powder shows evidence of damage, microbial contamination, or other signs of deterioration, such as powder caking or discoloration.
- Store reconstituted, sterilized complete media solutions at 2–8°C in a tightly sealed container protected from light.
- Do not use liquid solutions of sterilized complete media if they show evidence of large precipitation or other signs of deterioration.
- Use product within 18 months from date of manufacture listed on the product label.
- Prepare complete media in purified water such as water for injection (WFI) or equivalent.

Reconstitute using membrane filtration

Reconstitute Bacto™ CD Supreme Fermentation Production Medium (FPM) to 36.22 g/L using the following steps:

1. Weigh out 36.22 g of medium powder.
2. Fill a clean flask with 1,000 mL of room temperature water for injection (WFI) or equivalent.
3. Add the powder medium to the flask and mix until dissolved.
4. Measure the pH at room temperature. A typical pH can range from pH 6.8–7.2. Do not adjust pH above 7.2.
5. Add a carbon source, glycerol (4 to 10 mL) or glucose (10 to 20 g) as needed depending on process needs. Mix well until dissolved.
6. Sterilize the solution by filtration through a 0.2 µm filter membrane. For processes where autoclaving is desired, see “Reconstitute using autoclave sterilization” on page 2.
7. Store solution at 2–8°C away from light.

Alternative procedure note: If a certain process requires a separate sterilization of carbon sources, the media can be reconstituted without addition of a carbon source. Sterile carbon source can be added from appropriate stocks, as per user process, if desired. Do not autoclave the carbon source combined with Bacto™ CD Supreme Fermentation Production Medium (FPM) . The carbon source must be sterilized separately and added post-sterilization.

Reconstitute using autoclave sterilization

Reconstitute Bacto™ CD Supreme Fermentation Production Medium (FPM) to 36.22 g/L using the following steps:

1. Weigh out 36.22 g of medium powder.
2. Fill a clean flask with 1,000 mL of room temperature water for injection (WFI) or equivalent.
3. Add the powder medium to the flask and mix until dissolved.
4. Sterilize the solution by autoclaving at 121°C for 15 minutes.
5. Let the solution cool to room temperature before proceeding to the next step.
6. If using glycerol as a carbon source, add 4–10 mL of sterile glycerol depending on your process needs. Mix well to dissolve.

   Alternatively, if using glucose as a carbon source, add an appropriate amount of separately sterilized glucose stock (e.g., 400 g/L) to the target, 10–20 g/L depending on your process needs. Mix well until dissolved. For example, add 25.6 mL of 400 g/L dextrose stock to target 10 g/L glucose concentration in the final media.
7. Store solution at 2–8°C away from light.

Recommended testing guidelines

Growth and Protein production in shake flask

This procedure highlights an example of a protein production application with IPTG (isopropyl-beta-D-thiogalactopyranoside) induction using a protein production host strain such as BL21(DE3).

1. Prepare shake flasks by adding an appropriate volume of reconstituted Bacto™ CD Supreme Fermentation Production Medium (FPM) with carbon source. Add appropriate selection antibiotic as needed (e.g., 100 µg/mL of ampicillin). Typical volumes may vary from 20–40 mL media in a 125–250 mL baffled shake flask, shaken at 300 rpm at 35–37°C.

2. Prepare bacterial culture according to standard protocols using appropriate antibiotic selection LB agar plates. Prepare a seed culture from a colony of appropriate volume (e.g., 20–40 mL) in Bacto™ CD Supreme Fermentation Production Medium (FPM) with the appropriate selection antibiotic, to be grown for 15-20 hours, from a colony or saline suspension prepared from the plate. Alternatively, the user can also add bacterial stock directly to the Bacto™ CD Supreme Fermentation Production Medium (FPM) to start the seed culture.

3. Shake Flask Scale Up: Inoculate shake flasks containing fresh Bacto™ CD Supreme Fermentation Production Medium (FPM) with standard seeding density. E.g., 0.5%–10% of the shake flask volume using the previously grown seed culture or as per your process.

4. Measure optical density, OD_{600}, at various time points, and induce culture with the required amount of IPTG as per process requirements. Typical induction OD may be 0.6–3.0 and IPTG concentration for induction could be 0.05–1 mM. It is suggested that the user determine the optimum induction OD and IPTG concentration for Bacto™ CD Supreme Fermentation Production Medium (FPM) .

5. Harvest culture as per user-determined process. It is recommended to evaluate protein production over the entire growth curve taking readings at multiple time points, post-induction (e.g., 4 hrs, 6 hrs, 10 hrs, 15 hrs, 24 hrs).

Growth and Plasmid production in shake flask

This procedure highlights an example of plasmid production using a recombinant host strain such as DH1, DH5a, etc. For other strains, additional supplementation may be required. See “Additional recommendations” on page 4.

1. Prepare shake flasks by adding an appropriate volume of reconstituted Bacto™ CD Supreme Fermentation Production Medium (FPM) with glucose or glycerol. Typical values can be 20–40 mL in a baffled 125–250 mL baffled shake flask. Add appropriate selection antibiotic as needed (e.g., 100 µg/mL of ampicillin).

2. Prepare bacterial culture according to standard protocols, using an appropriate antibiotic selection and LB agar plates. Prepare a seed culture of appropriate volume (20–40 mL) in Bacto™ CD Supreme Fermentation Production Medium (FPM) with the appropriate selection antibiotic and grow for 20-28 hours from a colony or saline suspension prepared from the plate. Alternatively, the user can also add bacterial stock directly to Bacto™ CD Supreme Fermentation Production Medium (FPM) to start the seed culture.

3. If scale-up is desired, inoculate the shake flasks containing fresh Bacto™ CD Supreme Fermentation Production Medium (FPM) with the appropriate selection antibiotic and typical seeding density (e.g., 0.5%–10% of the shake flask volume) using the previously grown seed culture.

4. Measure OD_{600} at different time points to evaluate growth.

5. Harvest the culture as per user-determined process. It is recommended to evaluate plasmid production at different time points starting at late exponential to mid-stationary phase, which typically occurs from 18–30 hours.

Additional recommendations

- Bacto™ CD Supreme Fermentation Production Medium (FPM) can support growth of various *E. coli* strains, including *thi-1* strains. Some recombinant strains may require additional supplementation, such as amino acids, depending on the auxotrophic requirement of the strain. Check the genotype of your strain to determine additional supplementation requirements (see Table 1 below for additional information). For example, strains such as DH10b, Top10, etc. will require L-leucine and/or L-isoleucine supplementation for optimal performance in Bacto™ CD Supreme Fermentation Production Medium (FPM) due to Δ (*ara-leu*).

Table 1 Suggested amino acid supplementation requirements for some recombinant strains.

Type	Suggested Supplementation	Concentration (g/L)
DH-1	None	—
BL21	None	
DH5α	None	
DH10b, Top10, or strains carrying Δ (*ara-leu*)	L-leucine	0.5 g/L
Stbl3, Stbl2, JM108, JM109, etc. or strains carrying Δ (*lac-proAB*)	L-leucine + L-proline	0.5 g/L (both)

- For cultures using glycerol as the carbon source, additional glucose may be required if excessive lag phase is observed. In general, additional glucose may be added at 2-5 g/L to the glycerol-containing media for faster growth.
- Bacto™ CD Supreme Fermentation Production Medium (FPM) can be scaled up to bioreactors in batch mode following typical seeding processes. Use Bacto™ CD Supreme Fermentation Production Medium (FPM) as the seed culture medium. Additional feed can be added as per user requirements. The medium itself cannot be used as a feed. Typical feeds consist of carbon source, magnesium salts, and trace metal salts, as referenced in literature.

Troubleshooting

Observation	Possible cause	Recommended action
Very low to no cell growth (OD_{600} <1) at 24 hrs OR arrested growth after initial growth.	The strain might have some supplementation requirement due to auxotrophy.	Some recombinant strains may require additional supplementation, such as amino acids depending on auxotrophic requirement of the strain. See Table 2 in "Additional recommendations" on page 4. In general, strains carrying Δ (*ara-leu*) will require L-leucine supplementation with Bacto™ CD Supreme Fermentation Production Medium (FPM) .
		Make sure to reconstitute the media as per reconstitution instructions and add appropriate carbon source (glucose/glycerol) as per instructions.
Very low to no cell growth (OD_{600} <1) at 24 hrs and medium is brown/amber in color.	If the medium has turned brown upon autoclaving, it is likely that the medium was autoclaved with the glucose/glycerol added.	Follow the reconstitution instructions for the autoclaving method. The glucose must be sterilized separately and added to the Bacto™ CD Supreme Fermentation Production Medium (FPM) after the medium has been autoclaved.
Excessive Lag phase (>10 hrs) in seed culture when inoculating from peptone media plate or frozen stock.	Strain used.	Additional glucose may be added at 2–5 g/L to the glycerol-containing media for faster growth.
	Plasmid of interest used.	Additional glucose may be added at 2–5 g/L to the glycerol-containing media for faster growth.
	Used only glycerol as a carbon source.	Additional glucose may be added at 2–5 g/L to the glycerol-containing media for faster growth.
Lower protein production.	Induction time and/or inducer concentration were incorrect.	Optimize the timing of induction and inducer (e.g., IPTG) concentration in Bacto™ CD Supreme Fermentation Production Medium (FPM) depending on the growth characteristics of your cell line in this medium.
Excessive Lag phase (>5 hrs) seen during bioreactor scale up.	Seed media is a peptone-containing media (LB, Terrific Broth, etc.).	Prepare the seed culture for the shake flask scale up or bioreactor scale up in Bacto™ CD Supreme Fermentation Production Medium (FPM) .

Limited product warranty

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Thermo Fisher Scientific | 50 NW 176th Street | Miami, Florida 33169 USA
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Revision	Date	Description
A.0	02 June 2021	New manual.

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