Starter Pak No. 1

Catalog Number 215366

Doc. Part No. GRA0011234 Pub. No. MAN0019106 Rev. B.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

Gibco™ Starter Pak No. 1 is not manufactured under GMP conditions and is for evaluation use only. When initially using Starter Pak No. 1, it is recommended that all five peptones be concurrently evaluated to determine the peptone(s) which are most optimal to your culture system.

Gibco™ peptones are recommended for use with mammalian or microbial cell cultures, as supplements and/or feeds, to enhance growth, protein production, and overall culture performance in batch or fed-batch processes.

Contents and storage

Table 1 Starter Pak No. 1, Cat. No. 215366

Contents	Amount	Storage
Difco™ Yeast Extract, ultra-filtered (UF)	100 g	2°C to 25°C. Store in a dry place. Protect from heat, sunlight, and wide temperature swings.
Bacto™ TC Yeastolate	100 g	
Difco™ TC Yeastolate, ultra-filtered (UF)	100 g	
Difco™ Phytone™ Supplement, ultra-filtered (UF)	100 g	
Bacto™ Yeast Extract, technical	100 g	

Procedural guidelines

- Peptone powders are hygroscopic and should be stored at 2–25 °C in a dry place away from heat, sunlight, and wide temperature swings.
- When the peptone 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 peptone stock solutions at 2–8 °C in a tightly sealed container and protected from light.
- Do not use liquid stock solutions of peptones if they show evidence of precipitation or other signs of deterioration.
- Refer to the product label for the expiration date of each powder supplement when stored properly.
- Prepare peptones in M9 Minimal Salts (2x), or in an equivalent solution.
- Further supplementation with carbohydrates, growth factors, mineral salts or any other microbial specific supplements can be added as required.

Mammalian cell culture application

Reconstitute peptones

Reconstitute the peptone powders to 100 g/L using the following instructions:

1. Weigh 10 g of peptone powder.
2. Fill a clean 250 mL flask with approximately 90 mL room temperature water for injection (WFI) or equivalent.
3. Add the peptone to the flask and mix until completely dissolved.
4. Once completely dissolved, bring volume to 100 mL with WFI or equivalent.
5. Sterilize the solution by filtering through a 0.2 µm filter membrane or by autoclaving.
6. Store solution at 2–8 °C.

Titration study–batch culture in shake flask

1. Prepare shake flasks by adding an appropriate volume of reconstituted peptone (100 g/L stock solution) and basal medium to the final concentrations shown in Table 2.

Table 2 Titration study experimental set up (mammalian cell culture)

Condition	Peptone concentration	Base medium	Peptone stock	Total volume
Concentration 1	1 g/L	49.5 mL	0.5 mL	50.0 mL
Concentration 2	3 g/L	48.5 mL	1.5 mL	50.0 mL
Concentration 3	6 g/L	47.0 mL	3.0 mL	50.0 mL
Concentration 4	9 g/L	45.5 mL	4.5 mL	50.0 mL
Base medium only (negative control)	—	50.0 mL	—	50.0 mL

Note: This table provides guidance for titration of one peptone; similar set ups can be used for all peptones. Add appropriate positive control if needed.

2. Prepare seeding cell culture according to standard protocols. If cells are being cultured in a peptone containing medium, wash cells once in sterile PBS and pellet by centrifugation. Prepare seeding cell suspension by re-suspending the cell pellet in either PBS or the base control medium (negative control medium).
3. Inoculate shake flasks with standard seeding density.
4. On days 0, 3, 5, 7, 10, and last day of cell culture, determine viable cell density and percent viability.
5. Adjust glucose and L-glutamine levels as appropriate for the base medium throughout the experiment.
6. Determine protein titer on various days of culture, including the last day, to determine cumulative protein production.

Mixture study–batch culture in shake flasks

The peptones can also be blended to determine if additional enhancement is possible. The top three performing peptones selected from the titration studies can be mixed as per Table 3 to further optimize performance. Table 4 provides instructions for the experimental set up using a 100 g/L stock solution. Additional blending strategies should be evaluated based upon the results from the titration studies or from the suggested blending study.

Table 3 Mixture study (mammalian cell culture)

Mixture	Peptone 1	Peptone 2	Peptone 3	Peptone concentration
Mixture 1	2.0 g/L	0.5 g/L	0.5 g/L	3.0 g/L
Mixture 2	2.0 g/L	2.0 g/L	2.0 g/L	6.0 g/L
Mixture 3	0.5 g/L	2.0 g/L	0.5 g/L	3.0 g/L
Mixture 4	0.5 g/L	0.5 g/L	2.0 g/L	3.0 g/L
Mixture 5	0.5 g/L	0.5 g/L	0.5 g/L	1.5 g/mL

Table 4 Mixture study experimental set up (mammalian cell culture)

Test condition	Peptone 1 stock	Peptone 2 stock	Peptone 3 stock	Base medium	Total volume
Mixture 1	1.0 mL	0.25 mL	0.25 mL	48.50 mL	50.0 mL
Mixture 2	1.0 mL	1.0 mL	1.0 mL	47.0 mL	50.0 mL
Mixture 3	0.25 mL	1.0 mL	0.25 mL	48.50 mL	50.0 mL
Mixture 4	0.25 mL	0.25 mL	1.0 mL	48.50 mL	50.0 mL
Mixture 5	0.25 mL	0.25 mL	.25 mL	49.25 mL	50.0 mL
Base medium only (negative control)	—	—	—	50.0 mL	50.0 mL

Microbial cell culture application

Reconstitution instructions

It is recommended the peptone powders be hydrated at 30 g/L using the following instructions:

1. Weigh 6 g of the peptone powder.
2. Fill a clean 500 mL flask with approximately 180 mL of room temperature prepared M9 Minimal Salts solution or equivalent.
3. Add the peptone to the flask and mix until completely dissolved.
4. Measure pH at room temperature and adjust to the optimum pH for the application. A typical pH can range from 6.5 to 7.54 depending upon the microorganism. Avoid excessive pH adjustments.
5. Once completely dissolved, bring the final volume to 200 mL with room temperature prepared M9 Minimal Salts solution or equivalent.
6. Sterilize the solution by autoclaving or filtering through a 0.2-µm filter membrane.
7. Store solution at 2–8 °C.

Titration study in shake tubes or flasks

A peptone screen should be designed so that each peptone is evaluated at a variety of concentrations. Typical working concentrations for peptones range from 5 g/L to 30 g/L and are dependent on the microorganism's requirements. Table 5 outlines a recommended peptone titration experimental study starting with a peptone stock of 30 g/L in buffered salt solution (M9 Minimal Salts or equivalent).

Table 5 Titration study set up (microbial culture)

Concentration	Peptone concentration	Buffered salt solution	Peptone stock	Total volume
Concentration–1	30 g/L	—	50.0 mL	50 mL
Concentration–2	20 g/L	16.7 mL	33.3 mL	50 mL
Concentration–3	10 g/L	33.3 mL	16.7 mL	50 mL
Concentration–4	5 g/L	41.7 mL	8.3 mL	50 mL

Mixture study in shake tubes or flasks

Blends of peptones should also be considered, because synergistic effects have been observed in some processes when multiple peptones are used. The top two performing peptones from the titration study can be blended as outlined in Table 6 and Table 7 starting with a stock concentration of 30 g/L in buffered salt solution (M9 Minimal Salts or equivalent).

Table 6 Mixture study (microbial culture)

Mixture	Peptone 1	Peptone 2	Peptone concentration
Mixture 1	15 g/mL	15 g/L	30 g/L
Mixture 2	12 g/mL	8 g/L	20 g/L
Mixture 3	10 g/mL	10 g/L	20 g/L
Mixture 4	8 g/mL	12 g/L	20 g/L
Mixture 5	5 g/mL	5 g/L	10 g/L

Table 7 Mixture experimental set up (microbial culture)

Test condition	Peptone 1 stock	Peptone 2 stock	Buffered salts solutions	Total volume
Mixture 1	25.0 mL	25.0 mL	—	50.0 mL
Mixture 2	20.0 mL	13.3 mL	16.7 mL	50.0 mL
Mixture 3	16.7 mL	16.7 mL	16.6 mL	50.0 mL
Mixture 4	13.3 mL	20.0 mL	16.7 mL	50.0 mL
Mixture 5	8.3 mL	8.3 mL	33.4 mL	50.0 mL

Related products

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Item	Source
Difco™ Yeast Extract, ultra-filtered (UF)	210929
Bacto™ Yeast Extract, technical	288620
Bacto™ TC Yeastolate	255772
Difco™ TC Yeastolate, ultra-filtered (UF)	292804
Difco™ Phytone™ Supplement, ultra-filtered (UF)	210931
Glucose, powder (1 kg)	15023021
Glucose Solution (50 mL)	A2494001
L-Glutamine (100 g)	21051024
Pluronic™ F-68 (Kolliphor™ P 188) (100 mL)	24040032
M9 Minimal Salts (2x)	A1374401
Cell line specific supplements as required	MLS

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For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.

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