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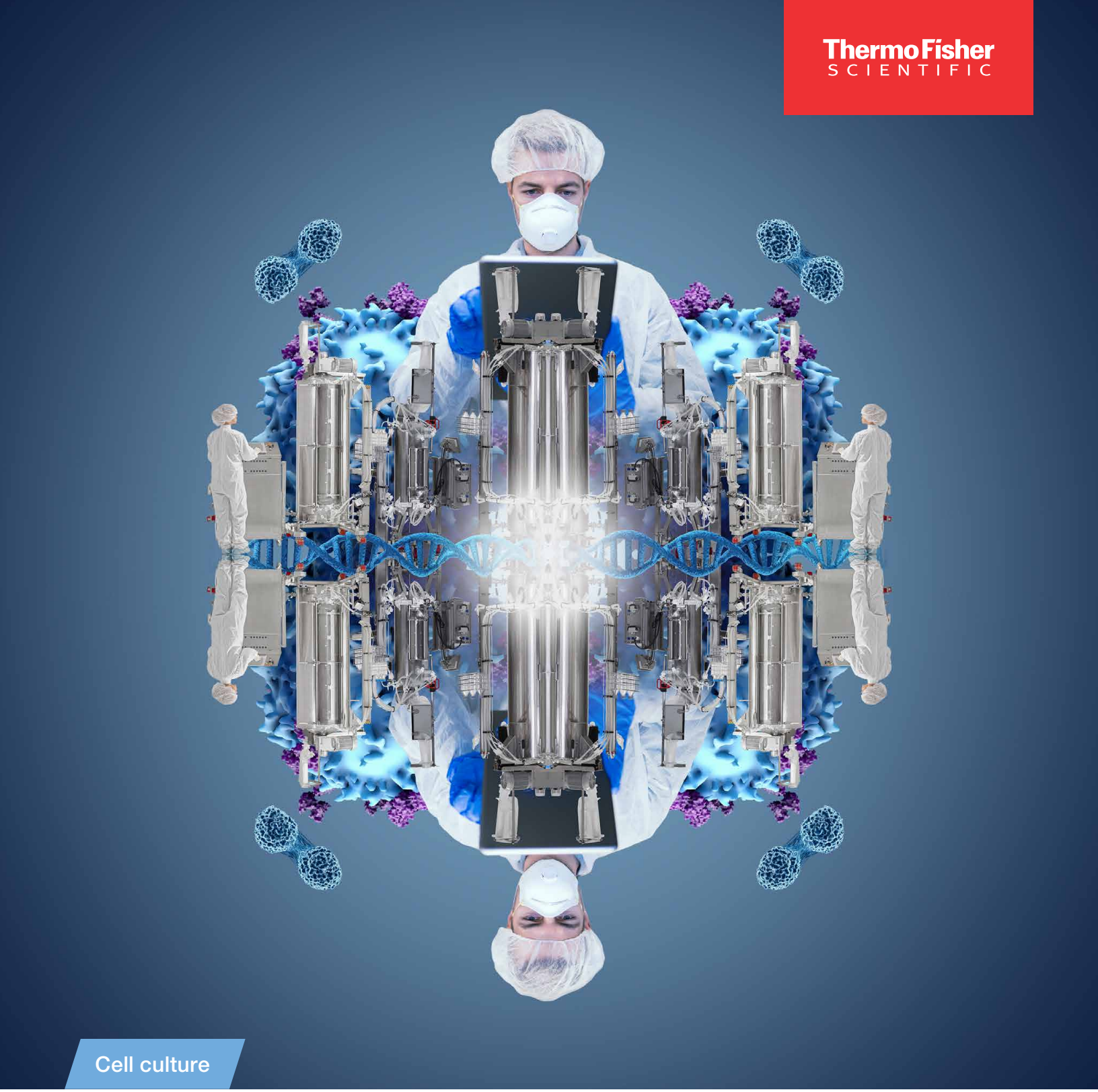


Cell culture

Screening peptones using Gibco Starter Paks

Including “titration and blending” protocol examples

gibco

Peptones have a long, proven history of being used to create robust, high-performance media and supplements. Given the unique nature of peptones and their diverse nutritional profiles, they can provide numerous protective benefits to a cell culture with the addition of just one supplement, including nutritional buffering and protection from toxic components present in a medium or process. This allows maintenance of high concentrations of key components and delay of apoptosis.

Adding the right peptone to your process can dramatically improve the culture environment to achieve production goals. But selecting the optimal peptone for your culture should take into consideration the cell type and application, followed by a peptone screening process.

Considerations for selecting peptones to screen

Given the availability of a wide variety of peptones, it is important to identify those that have been specifically developed for your application, as well as to characterize the key drivers within your process that affect culture performance.

For mammalian cells, it is important to start the screening as early as possible in the optimization process so that the right peptone is selected and optimized along with the base medium. While any medium can be enhanced with peptone additions, a more enriched base medium generally yields better results than a deficient medium.

For optimal growth, microorganisms generally need sufficient sources of carbon, nitrogen, inorganic phosphate and sulfur, trace metals, and vitamins, which are readily available in most peptones. Peptones for microbial applications act as the major source of nutrition and buffering within the culture, and are generally used at concentrations up to 30 g/L. Since they are such a critical component of microbial media, screening must be done to identify the peptones that will meet the unique needs of an organism. For example, anaerobes and aerobes have different nutritional preferences.

While considering peptones for both mammalian and microbial cultures, it is important to screen a wide variety of peptones from multiple sources as well as products from the same source, since they will all have different nutritional profiles.

The importance of peptone screening is to find the ideal profile to complement your culture and media to achieve your production goals.

To help simplify the peptone screening process, Thermo Fisher Scientific offers Gibco™ Starter Paks, which are ready-to-test sample packs of commonly used peptones for specific applications, including the production of monoclonal antibodies, recombinant proteins, and vaccines for both human and animal health applications.

Starter Paks

Gibco™ Starter Paks were created with your peptone screening process in mind. With 100 g samples conveniently packaged together, you can identify the correct supplements for your specific cell lines faster.

Ultrafiltered peptones ideal for human health applications

Gibco™ Starter Pak No. 1* features a combination of yeast- and soy-based peptones. Three of the products in this pack have been ultrafiltered to reduce endotoxin levels. The yeast products add a mixture of peptides, amino acids, carbohydrates (simple and complex), nucleosides, and vitamins to any medium formulation. All of these products have been successfully used in human and animal health applications.

- **Gibco™ Difco™ TC Yeastolate UF**
  Gibco™ Bacto™ TC Yeastolate
  These peptones are ideal for CHO cell–based applications of biotherapeutic monoclonal antibodies and recombinant proteins.
- **Gibco™ Difco™ Yeast Extract UF**
  Gibco™ Bacto™ Yeast Extract, Technical
  These peptones support optimal growth of many microbial species for a variety of human and animal health vaccines.
- **Gibco™ Difco™ Phytone Supplement UF**
  This enzymatic digest of soy is an excellent, nutritious source of carbohydrates and is used in mammalian cell culture. This peptone works well alone and when blended with yeast-based peptones.

Animal origin–free and animal-origin peptones best suited for vaccine production

Gibco™ Starter Pak No. 2* offers many essential nutrients needed for the production of human and animal vaccines.

- **Gibco™ Bacto™ Yeast Extract**
  This yeast has the highest level of carbohydrates among our yeast products and works well in a variety of human and animal health vaccines.
- **Gibco™ Phytone™ Peptone**
  Gibco™ Difco™ Soytone
  Both enzymatic digests of soy, these peptones are also nutritious sources of carbohydrates. These products work well in microbial fermentation processes as well as in mammalian cell culture processes such as those using CHO cells. Blending these soy peptones with yeast peptones has been shown to provide additional benefit to cultures.
- **Gibco™ Bacto™ Proteose Peptone No. 2**
  Gibco™ Bacto™ Proteose Peptone No. 3
  These enzymatic digests of porcine protein provide nutrition for fastidious microorganisms. Bacto Proteose Peptone No. 3 can replace serum in many applications and helps increase monoclonal antibody and recombinant protein production in CHO cells.
- **Gibco™ Bacto™ Casamino Acids**
  This supplement has low salt and iron content, making it an excellent supplement for media formulations for which nitrogen requirements are minimal.

Animal origin–free peptones for animal and human vaccine production

Gibco™ Starter Pak No. 3* provides a variety of yeast and soy products, ideal for processes when an animal origin–free medium is preferred.

- **Gibco™ Bacto™ Yeast Extract**
  Gibco™ Yeast Extract
  These peptones contain a mixture of peptides, amino acids, carbohydrates, and vitamins to support optimal growth of microbial species, and are ideal for the production of vaccines.
- **Gibco™ Bacto™ TC Yeastolate**
  This peptone is ideal for CHO cell–based applications of biotherapeutic monoclonal antibodies and recombinant proteins, as well as vaccine applications.
- **Gibco™ Phytone™ Peptone**
  Gibco™ Difco™ Soytone
  These soy-based supplements are nutritious sources of carbohydrates for successful use in microbial fermentation. Blending these soy peptones with yeast peptones has been shown to provide additional benefit to cultures.
- **Gibco™ Bacto™ Malt Extract**
  A water-soluble portion of malted barley, this peptone also provides carbohydrates for a variety of microbial fermentation processes.

Ordering information

Product name	Cat. No.
Starter Pak No. 1	215366
Starter Pak No. 2	215367
Starter Pak No. 3	215368

* Starter Paks are non-GMP and are used for evaluation purpose only.

Titration and blending protocols

To identify the ideal peptone for your production goals, each Starter Pak peptone should be titrated to identify and evaluate its components and their concentrations. Starter Paks are an ideal starting point in your screening, as they offer a wide variety of peptones from multiple sources, as well as products from the same source, which are critical since all cell lines will have different nutritional profiles. Synergistic effects have also been observed when blending peptones, so additional methods for mixing components are also recommended. The following titration and blending protocols can be used as provided, or as a starting point for designing custom screening studies.

Peptone procedure

Mammalian cell culture application

Reconstitution instructions

For mammalian cell culture, hydrate peptone powders at 100 g/L using the following instructions:

- Weigh 10 g of Gibco peptone powder.
- To a clean 250 mL beaker, add approximately 90 mL of room-temperature water for injection (WFI) or equivalent. Add the Gibco peptone to the beaker and mix until completely dissolved.
- Once the contents are completely dissolved, bring the volume up to 100 mL with WFI or equivalent.
- Sterilize the solution by filtration through a 0.2 µm filter membrane or by autoclaving. Store the solution at 2–8°C.

Titration study—batch culture in shake flasks

Step 1: Prepare shake flasks by adding appropriate volumes of reconstituted Gibco peptone (100 g/L stock solution) and base medium to the final concentrations shown in Table 1. In addition to the components in Table 1, cultures should be supplemented with glucose, L-glutamine, Pluronic™ F 68 (Kolliphor™ P 188), and any other cell line–specific supplements as required.

Step 2: Prepare the 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 the seeding cell suspension by resuspending the cell pellet in base medium.

Step 3: Inoculate shake flasks at standard seeding density.

Step 4: On days 0, 3, 5, 7, 10, and the last day of cell culture, determine viable cell density and percent viability.

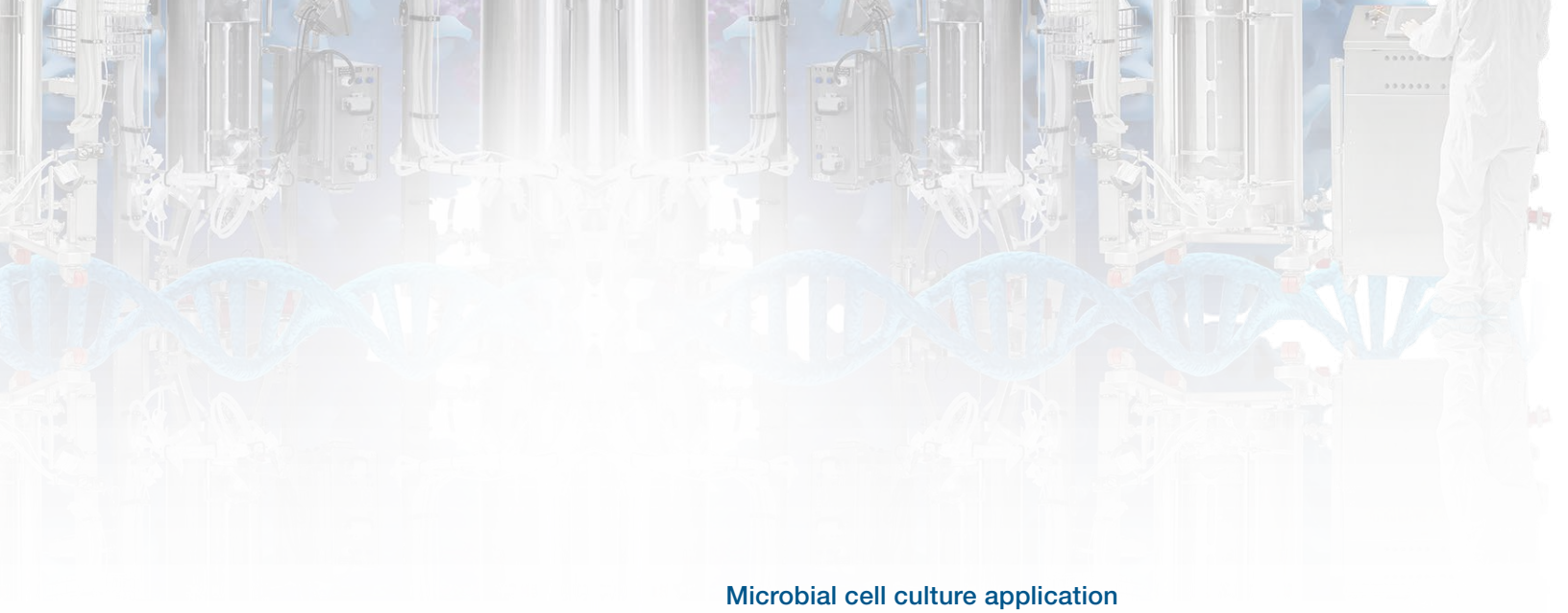
Step 5: Adjust glucose and glutamine levels as appropriate for the base medium, throughout the experiment.

Step 6: Determine protein titer on various days of culture, including the last day, to determine cumulative protein production.

Table 1. Titration study (mammalian cell culture).

Condition	Gibco peptone concentration (g/L)	Volume of base medium (mL)	Volume of Gibco peptone stock, 100 g/L (mL)
Concentration 1	1	49.5	0.5
Concentration 2	3	48.5	1.5
Concentration 3	6	47.0	3.0
Concentration 4	9	45.5	4.5
Medium only (negative control)	0	50.0	0

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



Mixture study—batch culture in shake flasks

The three top-performing Gibco peptones selected from the titration studies can be mixed as per Table 2 to further optimize performance. Table 3 provides recipes for the experimental setup using 100 g/L stocks.

Table 2. Mixture study (mammalian cell culture).

Mixture	Gibco peptone 1 (g/L)	Gibco peptone 2 (g/L)	Gibco peptone 3 (g/L)	Total peptone (g/L)
Mixture 1	2.0	0.5	0.5	3.0
Mixture 2	2.0	2.0	2.0	6.0
Mixture 3	0.5	2.0	0.5	3.0
Mixture 4	0.5	0.5	2.0	3.0
Mixture 5	0.5	0.5	0.5	1.5

Table 3. Experimental setup (mammalian cell culture).

Shake flask	Gibco peptone 1 stock (mL)	Gibco peptone 2 stock (mL)	Gibco peptone 3 stock (mL)	Volume of base medium (mL)
Mixture 1	1	0.25	0.25	48.50
Mixture 2	1	1	1	47.00
Mixture 3	0.25	1	0.25	48.50
Mixture 4	0.25	0.25	1	48.50
Mixture 5	0.25	0.25	0.25	49.25
Control	0	0	0	50

Microbial cell culture application

Reconstitution instructions

For microbial cell culture, hydrate peptone powders at 30 g/L using the following instructions:

- Weigh 6 g of Gibco peptone powder.
- To a clean 500 mL beaker, add approximately 180 mL of room temperature WFI, deionized (DI) water, or equivalent purified water.
- Add the Gibco peptone to the beaker and mix until completely dissolved.
- Once the contents are completely dissolved, bring the volume up to 200 mL with WFI, DI water, or equivalent.
- Measure the pH at room temperature and adjust to between 6.5 and 7.4. Avoid excessive pH adjustments. Sterilize the solution by filtration through a 0.2 µm filter membrane or by autoclaving.
- Store the solution at 2–8°C.

Titration study in shake tubes or flasks

A screening experiment should be designed so each Gibco peptone is evaluated at a variety of concentrations. Typical working concentrations for Gibco peptones range from 5 g/L to 30 g/L and are dependent on the microorganism's need. Table 4 outlines a recommended peptone titration experimental study. In addition to the components in Table 4, additional supplementation may be required to support growth of microorganisms, such as buffer salts like phosphates or a base medium like M9 Minimal Salts (Millipore Sigma). Further supplementation with carbohydrates, like glucose; growth factors, like serum or blood; mineral salts, like magnesium, calcium, or iron; and any other microbe-specific supplements can be added as required for peptone optimization.

Mixture study in shake tubes or flasks

Blends of Gibco peptones should also be considered because synergistic effects can be observed in some processes when multiple Gibco peptones are used. The two top-performing Gibco peptones from the titration study can be blended as outlined in Tables 5 and 6. Selection of the Gibco peptone or blend of Gibco peptones should be based on both cell proliferation and production data.

Table 4. Titration study (microbial cell culture).

Concentration	Gibco peptone (g/L)	Volume of buffer salts or purified water (mL)	Volume of Gibco peptone stock, 30 g/L (mL)	Total volume (mL)
Concentration 1	30	0.0	50.0	50
Concentration 2	20	16.7	33.3	50
Concentration 3	10	33.3	16.7	50
Concentration 4	5	41.7	8.3	50

Table 5. Mixture study (microbial cell culture).

Mixture	First Gibco peptone concentration (g/L)	Second Gibco peptone concentration (g/L)	Total Gibco peptone concentration (g/L)
Mixture 1	15	15	30
Mixture 2	12	8	20
Mixture 3	10	10	20
Mixture 4	8	12	20
Mixture 5	5	5	10

Table 6. Instructions for experimental setup (microbial cell culture).

Mixture	Volume of first Gibco peptone stock in buffer salts or purified water (mL)	Volume of second Gibco peptone stock in buffer salts or purified water (mL)	Volume of buffer salts or purified water (mL)	Total volume (mL)
Mixture 1	20.0	13.3	16.7	50
Mixture 2	16.7	16.7	16.7	50
Mixture 3	13.3	20.0	16.7	50
Mixture 4	8.3	8.3	33.3	50
Mixture 5	25.0	25.0	0	50

To see the complete list of peptones we offer and start your screening now, visit **thermofisher.com/peptones**

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