

# Expi293™ Met (-) Expression Medium

Catalog Number A4096701

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 **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](http://thermofisher.com/support).

## Product description

Expi293™ Met (-) Expression Medium is an optimized, chemically defined, serum-free, animal origin-free (AOF) formulation that is designed to support methionine labeling work and transfection of 293 cells (such as Expi293F™ cells) in suspension. The medium does not contain any methionine, protein, undefined lysates, or components of animal origin.

Expi293™ Met (-) Expression Medium is formulated with GlutaMAX™ Supplement, and only requires methionine supplementation. The medium is not recommended for adherent 293 cell culture.

## Contents and storage

Content	Amount	Storage	Shelf life <sup>[1]</sup>
Expi293™ Met (-) Expression Medium	1 L	2–8°C Protect from light	12 months

<sup>[1]</sup> Shelf Life duration is determined from Date of Manufacture.

## Culture conditions

**Media:** Expi293™ Expression Medium for culture. Expi293™ Met (-) Expression Medium for protein labeling.

**Cell line:** Expi293F™ cell lines

**Culture type:** Suspension

**Shake flask type:** Use PETG or polycarbonate, non-baffled, vented Erlenmeyer flasks; however, baffled Erlenmeyer flasks can also be used. Cultures can be scaled up in spinner flasks or bioreactors.

**Temperature range:** 37°C ±0.5°C

**Shaker speed:** For shakers with a 19-mm shaking diameter, set the shake speed to 125 ±5 rpm. For shakers with a 25-mm shaking diameter, set the shake speed to 120 ±5 rpm. For shakers with a 50-mm shaking diameter, set the shake speed to 95 ±5 rpm.

**Incubator type:** ≥80% humidified, 8% CO<sub>2</sub> atmosphere. Ensure proper gas exchange and minimize exposure of culture to light.

## Prepare media

- Expi293™ Met (-) Expression Medium contains GlutaMAX™ Supplement and does not require further supplementation with L-glutamine or GlutaMAX™ Supplement.
- Expi293™ Met (-) Expression Medium requires supplementation with methionine. We recommend a final concentration of 225 mg/L for L-Methionine (Methyl-<sup>13</sup>C) or 50 mg/L for L-Selenomethionine during protein labeling experiments.
- Expi293™ Met (-) Expression Medium is sensitive to light. Use and store the medium protected from light.
- Antibiotics are not recommended. However, 5 mL/L of Antibiotic-Antimycotic (Cat. No. 15240) containing penicillin, streptomycin, and amphotericin B can be used when required.
- Expi293F™ Cells should be thawed, recovered, and maintained in Expi293™ Expression Medium (Cat. No. A1435101). At the time of protein labeling, Expi293™ Expression Medium is replaced with Expi293™ Met (-) Expression Medium.

## Thaw Expi293F™ cells

1. Remove the vial of cells from liquid nitrogen, then swirl in a 37°C water bath for 1 to 2 minutes to thaw the cells rapidly until only a small amount of ice remains.

Do not submerge the vial in the water.

2. Just before the cells are completely thawed, decontaminate the vial by wiping it with 70% ethanol before opening it in a laminar flow hood.
3. Use a 2-mL or 5-mL pipette to transfer the entire contents of the cryovial into a 125-mL PETG or polycarbonate, disposable, sterile, vented Erlenmeyer shake flask containing 30 mL of pre-warmed Expi293™ Expression Medium (Cat. No. A1435101).
4. Incubate the cells in a 37°C incubator with ≥80% relative humidity and 8% CO<sub>2</sub> on an orbital shaker platform according to the following table.

Shaker diameter	Shake speed (rpm)
19 mm	125 ± 5
25 mm	120 ± 5
50 mm	95 ± 5

5. Allow cells to culture for 3–4 days post-thaw, then determine viable cell density and percent viability.

**Note:** At 24 hours post-thaw, viability can drop to ~80%, but should not get below 70%. It can take up to 7 days for cells to recover and reach ≥90% viability post-thaw.

6. Perform the first subculture when the viable cell density reaches 1–3 × 10<sup>6</sup> viable cells/mL (typically 4–7 days post-thaw).

## Subculture Expi293F™ cells

1. Use the viable cell density to calculate the volume of cell suspension required to seed a new shake flask according to the recommended seeding densities in Table 1 and the recommended culture volumes in Table 2.

**Table 1** Recommended seeding densities for routine cell culture maintenance

Sub-culture timing	Recommended seeding density
For cells ready 3 days post-subculture	0.4–0.5 × 10 <sup>6</sup> viable cells/mL
For cells ready 4 days post-subculture	0.3–0.4 × 10 <sup>6</sup> viable cells/mL

**Table 2** Recommended volumes for routine cell culture maintenance in vented, non-baffled flask

Flask size	Culture volume (mL)	Shake speed
125 mL	30–35 mL	125 ± 5 rpm (19 mm shaking diameter)
250 mL	60–70 mL	
500 mL	100–120 mL	120 ± 5 rpm (25 mm shaking diameter)
1 L	220–240 mL	95 ± 5 rpm (50 mm shaking diameter)
2 L	440–480 mL	
3 L	800–1,000 mL	90 ± 5 rpm
		85 ± 5 rpm
		80 ± 5 rpm

2. Transfer the calculated volume of cells to fresh, pre-warmed Expi293™ Expression Medium in a shake flask.
3. Incubate flasks in a 37°C incubator with ≥80% relative humidity and 8% CO<sub>2</sub> on an orbital shaker platform until cultures reach a density of 3–5 × 10<sup>6</sup> viable cells/mL.

**Note:** Do not let cells grow above 5 × 10<sup>6</sup> viable cells/mL during routine culture.

**Note:** Cells that are subcultured at densities outside of this early log-phase growth window can show longer doubling times and lower protein titers over time. Modify the initial seeding density to attain the target cell density of 3–5 × 10<sup>6</sup> viable cells/mL at the time of subculturing.

4. Repeat step 1 to step 3 to maintain or expand the cells for transfection.
5. The Expi293F™ Cells can now be transferred to Expi293™ Met (-) Expression Medium in preparation for protein labeling.

For detailed instructions on performing methionine protein labeling in Expi293F™ Cells, refer to the *Expi293™ Expression System User Guide* (MAN0007814) at [thermofisher.com](http://thermofisher.com).

## Related products

Product	Cat. No.
Expi293F™ Cells (1 × 10 <sup>7</sup> cells/vial)	A14527
Expi293F™ Cells, 6 vial "Cell Bank" pack (1 × 10 <sup>7</sup> cells/vial)	A14528
Expi293™ Expression Medium	A1435101
Expi293™ Met (-) Protein Labeling Kit	A41249
ExpiFectamine™ 293 Met (-) Transfection Kit	A39249
Opti-Plex™ Complexation Buffer	A4096801
Nalgene™ Single-Use PETG Erlenmeyer Flasks with Plain Bottom: Sterile	4115-0125
L-Selenomethionine	A39247
L-Methionine (Methyl- <sup>13</sup> C)	A39248

## Explanation of symbols

The symbols present on the product label are explained in the following table.

	MANUFACTURER		USE BY
	PROTECT FROM LIGHT		CONSULT INSTRUCTIONS FOR USE
	CATALOG NUMBER		CAUTION, CONSULT ACCOMPANYING DOCUMENTS
	BATCH CODE		UPPER AND LOWER LIMITS OF TEMPERATURE
	Sterilized using aseptic processing technique		

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For descriptions of symbols on product labels or product documents, go to [thermofisher.com/symbols-definition](http://thermofisher.com/symbols-definition).

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Revision	Date	Description
A.0	14 April 2019	New document.

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