HyClone™ Peak Expression Medium

Instructions for Use

Product description

HyClone™ peak expression medium (the product) is intended to be used for both growth and transfection of human embryonic kidney cells (HEK293) for adeno-associated virus (AAV) production.

Peak expression medium is an animal-derived component-free (ADCF), hydrolysate-free, and serum-free cell culture medium. This product contains a stable form of glutamine and poloxamer 188, and supports for high-yield virus production and increased cell culture efficiency.

This product is available as ready-to-use liquid medium, or as powder medium.

Safety

For use and handling of the product, refer to the Safety Data Sheet on *cytiva.com*.

Storage

Liquid and powder medium should be stored at 2°C to 8°C in a dry environment, protected from light. In addition, powder medium should be stored in a tightly sealed container to protect from moisture.

Shelf life

Liquid medium is stable up to 12 months and powder medium is stable up to 24 months from the date of manufacture, when stored at 2°C to 8°C and protected from light.

Note: *Refer to the product label for the expiry date.*

Disposal

Dispose contents and container in accordance with all local and national regulations.

Specifications and components

The specifications of this product are as follows:

- ADCF formulation
- Hydrolysate-free
- Serum-free

The components for the liquid and powder medium are listed below.

Components	Liquid medium	Powder medium
Alanyl-glutamine (4 mM)	+	+
Poloxamer 188 (0.08%)	+	+
Sodium bicarbonate	+	-
Phenol red	-	-

Required materials for hydration of powder medium

The following equipment and materials, not provided with the product, are required to hydrate the powder medium.

- Mixing vessel
- Stirrer

  Note: *A magnetic stirrer can be used for small-scale hydration up to 5 L. An overhead or bottom-mounted impeller is recommended for larger volumes.*
- Calibrated pH meter
- Calibrated osmometer
- Cell culture-grade water
- Sodium bicarbonate
- 0.2 µm filter
- Container for hydrated medium

Hydration of powder medium

Follow the steps below to hydrate the powder medium.

Step	Action
1	Fill a clean mixing vessel with 90% of the final volume with cell culture-grade water at ambient temperature (20°C to 25°C). For example, start with 900 mL of water for a preparation of 1 L medium. Start stirring.
2	Slowly add 25.17 g/L powder to the vessel to avoid clumping. Mix for 20 minutes until dissolved.
3	Slowly add 2.50 g/L sodium bicarbonate. Mix for 10 minutes until all components are completely dissolved. *Result:* The medium should be a pale yellow and particulate-free solution. Prior to pH adjustment, the medium can be slightly hazy.

Step	Action
4	Adjust the pH to 7.2 (acceptable range pH 7.0 to 7.4) by drop-wise addition of 5 N NaOH or HCl.

NOTICE
Use caution when adjusting pH. Over adjusting can cause the osmolality to be out of specification.

5	Adjust the medium to the final volume with cell culture-grade water at ambient temperature (20°C to 25°C).
6	Measure and record the final pH and osmolality. Final values must be: • pH 7.0 to 7.4 • Osmolality 300 to 340 mOsm/kg
7	Sterile filter the medium immediately after hydration using a 0.2 µm filter.
8	Store the hydrated medium in a container, protected from light at 2°C to 8°C until use.

General culture recommendations

For optimal culture performance, cultures should be incubated at 37°C in a 5% CO_2 environment.

Cell adaptation

Adaptation of HEK293 cells from another medium to this product can be achieved by two methods:

- Direct adaptation
- Gradual adaptation

Follow the recommendations below for direct or gradual adaptation of HEK293 cells.

Direct adaptation

- Seed suspension HEK293 cells from the current medium to this product at a cell density of at least 3×10^5 viable cells/mL. Increasing the seeding density 2 to 3 times (i.e., 6×10^5 viable cells/mL or 9×10^5 viable cells/mL) can be beneficial.
- Passage cells every 3 to 4 days.
- Passage cells 3 to 4 times in this product to make sure that the adaptation is complete. The cells should have at least 90% viability after 3 to 4 passages.
- If the cell viability does not reach 90% after 3 to 4 passages, consider switching to a gradual adaptation approach.
- If the cell density is low at scheduled passage intervals, consider waiting another 24 to 36 hours to passage.
- Make sure that cells are completely adapted to this product before performing screening and/or benchmark studies.

Gradual adaptation

- Mix the current medium and this product at a stepwise ratio of 90:10, 75:25, 50:50, 25:75, and 0:100.

  Tip: *Customized combinations can also be beneficial.*

- Seed suspension HEK293 cells from the current medium to this product at a cell density of at least 3×10^5 viable cells/mL. Increasing the seeding density 2 to 3 times can be beneficial at later ratios (50:50, 25:75, etc.).
- Make sure that cells are healthy and reach normal densities prior to moving to the next step of adaptation.
- Cells can be spun down to remove spent media prior to passage into fresh medium (optional).

  Note: *Conditioned media carried over during passaging can be beneficial for certain cell lines.*

- Passage cells 3 to 4 times in this product to make sure that the adaptation is complete. The cells should have at least 90% viability after 3 to 4 passages.
- Make sure that cells are completely adapted to this product before performing screening and/or benchmark studies.

Cryopreservation

Adapted cells can be cryopreserved in this product with 10% Dimethyl Sulfoxide (DMSO). The recommended cell density for freezing is a minimum of 1.0×10^7 viable cells/mL.

Quality control testing

Quality control test specifications are listed in the table below.

Parameter	Specification
Appearance	Powder medium: off-white powder Liquid medium: pale yellow solution
pH (at 20°C to 25°C)	7.0 to 7.4 (with $NaHCO_3$)
Osmolality	300 to 340 mOsm/kg H_2O
Sterility	No growth (bacteria or fungi)
Endotoxin	< 1.0 EU/mL
HPLC amino acid profile	Complies (80% to 120%) recovery

Refer to *Certificate of analysis* for actual results.

Ordering information

Product	Pack size	Product code
HyClone peak expression powder medium	5 L HDPE bottle	SH31192.01
	10 L HDPE bottle	SH31192.02
	50 L HDPE bottle	SH31192.03
	100 L poly bag/pail	SH31192.04
	500 L poly bag/pail	SH31192.05
	1000 L poly bag/drum	SH31192.06
HyClone peak expression liquid medium	1 L bottle	SH31193.02
	1 L bag	SH31193.08
	5 L bag	SH31193.09
	100 L bag	SH31193.10
	200 L bag	SH31193.11
	10 L bag	SH31193.12
	20 L bag	SH31193.13
	50 L bag	SH31193.14

Technical support

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