

HyClone™ boost expression supplements a and b

HYCLONE™ MEDIA AND SUPPLEMENTS

HyClone™ boost expression supplements a and b are intended for use in combination with HyClone peak expression or prime expression media to provide high yields of viral vectors in processes when using human embryonic kidney (HEK) 293 cell lines. The supplements are available as a convenient ready-to-use liquid format or as a dry powder concentrated format to be hydrated before use (Fig 1). HyClone boost expression supplements a and b are chemically defined (CD), animal-derived component-free (ADCF), and optimized for high-yield virus production. The supplements do not contain any growth factors (such as insulin), peptides, hydrolysates, or phenol red, supporting batch-to-batch consistency and increased cell culture process efficiency.

HyClone boost expression supplements are designed specifically for virus productivity improvement. They are suitable for biomanufacturing using HEK293 cell lines such as ELEVECTA™ transient, VPC 2.0, and other in-house developed HEK293 cell lines.



Fig 1. HyClone boost expression supplements a and b in liquid and dry powder formats.

Features and benefits

- Demonstrate > 2-fold increase for upstream virus titer across multiple cell lines and viral vector serotypes (Fig 2).
- CD and ADCF formulations.
- Ready to use in both liquid and powder formats.
- Consistent performance from small- to large-scale processes.
- Supplements a and b complement each other and are used together to achieve optimum performance¹.
- Detailed protocols for use to maximize output.

¹Supplements a and b should be added to the cultivation vessel as individual solutions and should not be mixed in advance as this may lead to precipitation. The recommended ratio for boost expression supplements to cell culture is 10% (v/v) for supplement a and 1% (v/v) for supplement b. The suggested time window for the supplements addition is 18 to 24 h post-transfection. The timing and ratio of the supplements can be adjusted for different cell lines to maximize performance.

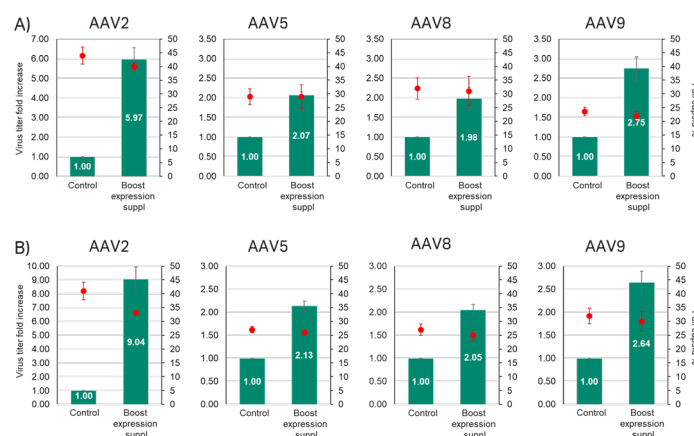


Fig 2. AAV virus titer improvement and percentage of full capsids when using HyClone boost expression supplements a and b with A) ELEVECTA transient cell line; B) VPC 2.0 cell line across multiple serotypes. Control experiments are using the same process condition without HyClone boost expression supplements.

Specifications

HyClone boost expression supplements specifications for liquid feed solution and dry powder formats:

- Protein-free
- Growth factor-free
- Phenol red-free
- Hydrolysate-free
- CD and ADCF formulated

HyClone boost expression supplement a

HyClone boost expression supplement a has a pH close to neutral and contains amino acids, vitamins, salts, trace elements, and glucose.

HyClone boost expression supplement b

HyClone boost expression supplement b has an alkaline pH and is a concentrated solution of amino acids.

Safety declaration

HyClone boost expression supplement b is a hazardous chemical:

- Causes skin irritation.
- Causes severe eye irritation.
- Can cause respiratory irritation.

Please take appropriate safety precautions prior to using HyClone boost expression supplement b.

1. Wear protective gloves.
2. Wear eye or face protection.
3. Avoid breathing dust.

If inhaled, move the individual to fresh air and keep at rest in a position comfortable for breathing. Call a poison center or physician if you feel unwell.

Store HyClone boost expression supplement b in a locked location.

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

Shelf life

HyClone boost expression supplements a and b liquid stored in PETE bottles have a shelf life up to 12 mo when stored at 2°C to 8°C.

HyClone boost expression supplements a and b liquid stored in bioprocess bags have a shelf life up to 9 mo when stored at 2°C to 8°C.

Please refer to the product label for the expiry date of your shipment.

Storage

Store HyClone boost expression supplements a and b at 2°C to 8°C, protected from light. In addition, powder supplements should be stored in a dry environment, protected from light, and stored at 2°C to 8°C.

Recommended protocol

1. Use HEK293 cells in the logarithmic phase of growth (3 to 6×10^6 cells/mL and viability $> 90\%$) that have been adapted into HyClone prime expression medium or HyClone peak expression medium.

2. Seed cells at 0.4×10^6 cells/mL 3 d before transfection (Day -3).

3. Transfect cells at Day 0 with a mixture of plasmid vectors and a transfection reagent, following the recommended protocol offered by the transfection reagent manufacturer. Cell viability at transfection should be $> 90\%$ with final viable cell density of 3×10^6 cells/mL considering the volume of the transfection complex.

Note:

Optimal transfection parameters vary based on several factors including but not limited to the cell type and DNA purity. Therefore, end-user specific optimization of parameters is recommended.

4. Add **10% (v/v) HyClone boost expression supplement a, and 1% (v/v) HyClone boost expression supplement b to the total culture volume 18 to 24 h post-transfection** (Day 1).

Note:

The HyClone boost expression supplements a and b should be added to the cultivation vessel as individual solutions and should not be mixed in advance as this may lead to precipitation. The recommended ratio of boost expression supplement a to boost expression supplement b is 10 to 1 (v/v).

5. Collect samples for cell count and viral titer measurement every day starting at Day 2 to find the optimum harvest time (usually Day 3 to 4).

Note 1:

We recommend performing metabolite measurements (pH, osmolality, L-glutamine, glucose, lactate, ammonium) at transfection (Day 0) and monitor them at harvest time.

Note 2:

We recommend sample collection (500 μ L/sample) at transfection and at harvest time for future spent media analysis.

HyClone boost expression supplements optimization guideline

HyClone boost expression supplements can be evaluated following the suggested application mentioned in the protocol. However, optimization of the feed schedule and volume is encouraged to accomplish maximum titers. The starting time of feeding should be at least 6 to 8 h post-transfection to avoid any interference with the transfection. The total feed can range 5% to 15% for HyClone boost expression supplement a, and 0.5% to 1.5% for HyClone boost expression supplement b; and can be applied as single additions or at multiple time points.

Note:

You should optimize feeding schedule and conditions, including concentration titration by monitoring cell health.



Custom production

Formulations and delivery systems can be customized to your specific process requirements or optimized to maximize process yields.

Rapid response production (RRP)

Our RRP program manufactures up to 200 L of your custom prototype formulation within seven working days of your request. Use our RRP service to expedite the development and testing of custom media for your biopharmaceutical manufacturing process.

Technical support

Our cell-culture medium specialists and technical support teams are available to discuss your specific cell culture requirements. In addition, we have an extensive service offering to help with, for example, process development, optimization, and scale-up. Please contact your local sales representative to learn more about the services we offer. To find a certificate or an MSDS for a specific product, please visit cytiva.com/certificates.

Ordering information

Product	Pack size ¹	Format	Product code
HyClone boost expression supplement a	500 mL bottle	Liquid	SH31207.01
	1000 mL bottle	Liquid	SH31207.02
	1 L bag ²	Liquid	SH31207.03
	5 L bag ²	Liquid	SH31207.04
	10 L bag ²	Liquid	SH31207.05

20 L bag ²	Liquid	SH31207.06
50 L bag ²	Liquid	SH31207.07
100 L bag ²	Liquid	SH31207.08
200 L bag ²	Liquid	SH31207.09

HyClone boost expression supplement a	0.5 L bottle ²	Powder	SH31206.01
	1 L bottle ²	Powder	SH31206.02
	5 L bottle ²	Powder	SH31206.03
	10 L bottle ²	Powder	SH31206.04

HyClone boost expression supplement b	100 mL bottle	Liquid	SH31209.01
	500 mL bottle	Liquid	SH31209.02
	1000 mL bottle	Liquid	SH31209.03
	1 L bag ²	Liquid	SH31209.04
	5 L bag ²	Liquid	SH31209.05
	10 L bag ²	Liquid	SH31209.06
	20 L bag ²	Liquid	SH31209.07

HyClone boost expression supplement b	0.5 L bottle	Powder	SH31208.01
	1 L bottle ²	Powder	SH31208.02
	5 L bottle ²	Powder	SH31208.03
	10 L bottle ²	Powder	SH31208.04

Related products	Pack size	Format	Product code
HyClone peak expression medium	1000 mL bottle	Liquid	SH31193.02
HyClone prime expression medium	1000 mL bottle	Liquid	SH31199.02

¹Powder product quantity is shown as the final volume of after powder reconstitution.

²Item is made to order. Lead times and minimum order quantities apply.

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