HyClone™ VaccineXpress cell culture medium for Vero cells

HyClone VaccineXpress cell culture medium is designed and developed for high-density growth and maintenance of kidney-derived cell lines (e.g., Vero cells) for viral vaccine manufacturing. VaccineXpress is serum-free (SF), animal-derived component-free (ADCF), and human origin-free. This medium has been qualified to grow attachment-dependent Vero cells with microcarriers for producing vaccines such as influenza, Zika, Dengue, and respiratory syncytial virus (RSV). The lack of serum and animal-derived components in the medium reduce variability of the process. It also enhances scalability and ease of purifying recombinant proteins and viruses in bioprocess applications. VaccineXpress is formulated without L-glutamine for extended shelf life. It is available in various configurations, in liquid and powder format (Fig 1).

VaccineXpress medium is well suited for applications ranging from multiwell plates to large-scale, microcarrier cultures in WAVE Bioreactor™ or Xcellerex™ bioreactors.

Key features of VaccineXpress medium

- SF, ADCF formulation
- Designed to support high peak cell density, viral infectivity, and productivity
- Allows for direct or sequential adaptation
- Designed to support microcarrier cultures
- Available in liquid and powder formats
- Suitable for small- to large-scale culture applications

Specifications

Liquid medium

- With sodium bicarbonate
- Without L-glutamine
- Without poloxamer 188
- Without phenol red

Powder medium

- Without sodium bicarbonate
- Without L-glutamine
- Without poloxamer 188
- Without phenol red

Fig 1. VaccineXpress cell culture medium is available as liquid or powder, in pack sizes suitable for small-volume cell culture as well as large-scale bioprocessing applications.

Product handling

This formulation (both powder and liquid) should be stored at 2–8°C, away from light.

Suggested preparation

Liquid medium

With the exception of L-glutamine addition, liquid medium is ready to use right out of the bottle. Add sterile-filtered L-glutamine. We recommend 4 mM final concentration.

Powder medium

1. While stirring, add 11.65 g/L powder medium to cell culture-grade water at 80% of final preparation volume. If your water source is normally cool, it is useful to adjust the water temperature. Using warmer room temperature water (20–25°C) will improve dissolution time. Mix 10 to 15 min or until dissolved.
2. Add sodium bicarbonate (2.0 g/L), and mix until dissolved. Adjust to pH 7.0 to 7.2 with HCl or NaOH as required.
3. Bring vessel to final volume with cell culture-grade water. Allow solution to mix for an additional 15 min.
4. Check pH and osmolality, and adjust if necessary. Expected values:
   - pH 7.0–7.2
   - Osmolality 280–310 mOsm/kg
5. Sterile filter the medium through a 0.22 µm sterilizing-grade filter into a desired container.

Preparation notes

L-glutamine can be added (4 mM final concentration) during step 2 if medium will be stored short term. For longer storage, we recommend adding L-glutamine at time of culture. Once hydrated, medium without L-glutamine can be stored at 2–8°C away from light for up to 6 months.

General culture recommendations

1. Incubate cultures at 37°C and in a 5% CO_2 environment.
2. Maintain adapted cells by establishing a mid-logarithmic growth phase subculturing schedule.
3. We suggest seeding cultures at a density of 3 to 5 × 10^4 cells/cm²; viability should be > 90%.
4. To maintain a completely serum-free and animal-free process, we recommend using recombinant trypsin (e.g., Sheffield™ rTrypsin ACF).
5. It is beyond the scope of this document to detail cell detachment procedures. Use your standard technique. Be aware that if you use trypsin, serum-free conditions necessitate use of a trypsin inhibitor, a cell wash step, or one (or more) medium exchanges.

Microcarrier culture considerations

VaccineXpress has been tested extensively to cultivate cells on GE Healthcare's Cytodex™ I Gamma microcarriers. Compatibility with other types of microcarriers is highly likely but has not been evaluated.

When using any type of microcarriers, we recommend adding 2.0 g/L poloxamer 188 as a shear protectant.

As is true with a normal serum-free static culture, detachment using trypsin requires neutralization with a soybean trypsin inhibitor (STI). Residual trypsin will negatively impact cell culture, but so will residual STI. Most STI manufacturers recommend using an equal volume (of a 1 mg/mL solution) to trypsin used, and this works well in static culture where all STI can easily be removed. However, in microcarrier culture we recommend using less STI (1:5 instead of 1:1 v:v), because of the difficulty in fully removing it.

Direct adaptation*

1. Transfer cells grown in current medium directly into VaccineXpress medium at 5.0 × 10^4 cells/cm².
2. When cell density reaches ~ 80% confluence, detach and subculture the cells in VaccineXpress medium and continue for two more passages.
3. Cells should be subcultured based on confluence, typically 72 to 96 h.
4. Direct adaptation generally works well with this medium; move to sequential adaptation if necessary.

Sequential adaptation*

Medium preparation: Mix medium currently being utilized with an equal volume of VaccineXpress medium. This constitutes a 50:50 mixture of original and new media.

1. Maintain a stock flask with the current acceptable growth rates. If adaptation to the new medium stalls, or falls to unacceptable growth rates, passage the stock one or two more times under its current conditions.
2. Subculture the cells into the mixture at a seeding concentration of 3–5 × 10^4 cells/cm². For best results, the culture should be ~ 80% confluent for adherent Vero cells and viability should be > 90%.
3. When the cells reach a density of ~ 80% confluence, subculture into another mixture of the original medium and fresh HyClone medium (25:75 original to new) at a seeding density of 3–5 × 10^4 cells/cm².
4. Continue passaging with decreased concentration of the original medium and increased concentration of new medium. After reaching a mixture concentration of 12.5% original medium, switching to straight VaccineXpress medium should work. Generally, cells will adapt within 3 to 4 passages (we recommend at least 6 population doublings).

*These procedures assume cells are already adapted to a serum-free medium. Adaptation from a serum-containing medium might require additional time and attention.

Cryopreservation

There are several cryopreservation and cryorecovery protocols; follow your internally recommended procedures. Adapted cells can be cryopreserved in VaccineXpress medium with 10% DMSO. We recommend freezing the cells at a minimum cell density of 1×10^7 cells/mL.

Quality control testing

Quality control test specifications are listed in Table 1.

Table 1. Test specifications[1]

Liquid medium	
Appearance	Clear solution
Osmolality	280 to 310 mOsm/kg
pH	7.0 to 7.2
Sterility	No growth (bacteria or fungi)
Endotoxin	< 1 EU/mL
Powder medium	
Appearance	Off-white powder
Endotoxin	< 10 EU/g
Growth promotion	Pass

Custom production

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

Rapid Response Production (RRP)

Use our non-cGMP RRP service to expedite the development and testing of custom media, buffers, and process liquids for your biopharmaceutical manufacturing process. RRP can manufacture up to 200 L or 20 kg of your custom prototype formulation within seven days of order.

Ordering information

VaccineXpress medium is manufactured in homogeneous liquid lot sizes up to 10 000 L and powder lot sizes up to 250 000 L.

Product description	Pack size	Product code
HyClone VaccineXpress powder medium*	10 L (HDPE bottle)	SH31127.01†
	50 L (HDPE bottle)	SH31127.02‡
	100 L (HDPE bottle)	SH31127.03‡
	500 L (Polybag/pail)	SH31127.04‡
HyClone VaccineXpress liquid medium	1000 mL (PETE bottle)	SH31126.01†
	10 L (bioprocess container)	SH31126.02‡
	20 L (bioprocess container)	SH31126.03‡
	50 L (bioprocess container)	SH31126.04‡
	100 L (bioprocess container)	SH31126.05‡

Related products	Pack size	Product code
L-glutamine 200 mM	100 mL bottle	SH30034.01†
	500 mL bottle	SH30034.02†
L-glutamine powder	500 g	SH30336.03‡

* Packaging has powder sufficient to make liquid medium equivalent to volume stated on the label.
† Item in stock.
‡ Item is made to order. Lead times and minimum order quantities apply.

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