



# Vero-SF-ACF

CCL-81.5™

## Description

Vero-SF-ACF is a cell line exhibiting epithelial morphology that was adapted to serum-free and animal component-free medium. This cell line can be used in the detection of verotoxin, efficacy testing, malaria biology, media testing, and mycoplasma testing.

**Organism:** *Cercopithecus aethiops*

**Age:** adult

**Morphology:** epithelial

**Growth properties:** Adherent

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## Storage Conditions

**Product format:** Frozen

**Storage conditions:** Vapor phase of liquid nitrogen

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## Intended Use

This product is intended for laboratory research use only. It is not intended for any animal or human therapeutic use, any human or animal consumption, or any diagnostic use.

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## BSL 1

ATCC determines the biosafety level of a material based on our risk assessment as guided by the current edition of *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, U.S. Department of Health and Human Services. It is your responsibility to understand the hazards associated with the material per your organization's policies

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and procedures as well as any other applicable regulations as enforced by your local or national agencies.

ATCC highly recommends that appropriate personal protective equipment is always used when handling vials. For cultures that require storage in liquid nitrogen, it is important to note that some vials may leak when submerged in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vial exploding or blowing off its cap with dangerous force creating flying debris. Unless necessary, ATCC recommends that these cultures be stored in the vapor phase of liquid nitrogen rather than submerged in liquid nitrogen.

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### Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at [www.atcc.org](http://www.atcc.org).

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### Growth Conditions

**Temperature:** 37°C

**Atmosphere:** 95% Air, 5% CO<sub>2</sub>

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### Handling Procedures

#### Unpacking and storage instructions:

1. Check all containers for leakage or breakage.
2. Remove the frozen cells from the dry ice packaging and immediately place the cells at a temperature below -130°C, preferably in liquid nitrogen vapor, until

ready for use.

### Complete medium:

These cells may be grown in NutriVero Flex10 (Biological Industries, Cat # 05-068-1A); plus 4mM L-Glutamine (ATCC 30-2214).

### Handling Procedure:

The flask was seeded with cells (see specific batch information) grown and completely filled with medium at ATCC to prevent loss of cells during shipping.

1. Upon receipt visually examine the culture for macroscopic evidence of any microbial contamination. Using an inverted microscope (preferably equipped with phase-contrast optics), carefully check for any evidence of microbial contamination. Also check to determine if the majority of cells are still attached to the bottom of the flask; during shipping the cultures are sometimes handled roughly and many of the cells often detach and become suspended in the culture medium (but are still viable).
2. **If the cells are still attached**, aseptically remove all but 5 to 10 mL of the shipping medium. The shipping medium can be saved for reuse. Incubate the cells at 37°C in a 5% CO<sub>2</sub> in air atmosphere until they are ready to be subcultured.
3. **If the cells are not attached**, aseptically remove the entire contents of the flask and centrifuge at 125 x g for 5 to 10 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 mL of this medium and add to 25 cm<sup>2</sup> flask. Incubate at 37°C in a 5% CO<sub>2</sub> in air atmosphere until cells are ready to be subcultured.

### Subculturing procedure:

Volumes used in this protocol are for a 75 cm<sup>2</sup> flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with Ca<sup>++</sup>/Mg<sup>++</sup> free Dulbecco's phosphate-buffered saline (D-PBS).
3. Add 2.0 to 3.0 mL of 0.25% (w/v) Trypsin-0.53mM EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).

**Note:** To avoid clumping do not agitate the cells by tapping or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may

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be placed at 37°C to facilitate dispersal.

4. Add 2.0 to 3.0 mL of 0.25% Soybean Trypsin Inhibitor and aspirate cells by gently pipetting.
5. Transfer cell suspension to a centrifuge tube and spin at approximately 125 x *g* for 5 to 10 minutes.
6. Discard supernatant and resuspend cells in fresh growth medium. Add appropriate aliquots of cell suspension to new culture vessels.
7. Incubate cultures at 37°C

### Note:

1. Cells grow best after the first subculture.
2. For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 13 in **Culture Of Animal Cells: A Manual Of Basic Technique** by R. Ian Freshney, 6th edition, published by Wiley-Liss, N.Y., 2010.

**Cell density:** The recommended start-up seeding range is  $3.0 \times 10^4$  to  $5.0 \times 10^4$  viable cells/cm<sup>2</sup> and the recommended subculture seeding range is  $2.0 \times 10^4$  to  $5.0 \times 10^4$  viable cells/cm<sup>2</sup>.

**Medium Renewal:** One to two times weekly

**Reagents for cryopreservation:** Stem Cell Freezing Medium (ATCC ACS-3020)

**Note:** Cell counts to determine freeze volume must be taken prior to centrifugation as the freeze medium is not compatible with trypan blue cell counting equipment.

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## Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: Vero-SF-ACF (ATCC CCL-81.5)

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## References

References and other information relating to this material are available at [www.atcc.org](http://www.atcc.org).

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## Contact Information

ATCC

10801 University Boulevard

Manassas, VA 20110-2209

USA

US telephone: 800-638-6597

Worldwide telephone: +1-703-365-2700

Email: [tech@atcc.org](mailto:tech@atcc.org) or contact your local distributor

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