




Product Sheet


# Trypsin-EDTA Solution, 1X (ATCC® 30-2101™)

Please read this **FIRST**



Storage Temp.  
**Store Trypsin-EDTA solution frozen, between -5°C and -20°C.**  
**Avoid repeated freeze-thaws by dispensing and storing in aliquots.**

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Biosafety Level  
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## Description

**Product Description:** 0.25% Trypsin/0.53 mM EDTA in Hanks Balanced Salt Solution without calcium or magnesium. For dissociation of cell monolayers. Trypsin-EDTA solution is suitable for most but not for all adherent cell lines. For cell line-specific information, please go to the appropriate product page on the web, refer to the product sheet supplied with the cell line, or contact ATCC Technical Service.

**Volume:** 100 mL

## Directions for Use

The amounts used in this procedure are for a 75 cm<sup>2</sup> flask. Adjust volumes as appropriate for different sized vessels.

1. Bring ATCC® Trypsin-EDTA solution to the appropriate temperature (see cell line product sheet). This may be 4°C, room temperature, or 37°C depending upon the cell type. You may also need to use a balanced salt solution [e.g., ATCC® Dulbecco's Phosphate Buffered Saline (PBS) without Ca or Mg, Catalog number 30-2200] to rinse the cells. If so, bring this to the same temperature. Finally, bring fresh, complete cell culture media to the appropriate temperature for cell growth (e.g., 37°C).
2. Remove and discard the cell culture medium from the flask.
3. Depending upon the cell line, rinse the cell monolayer with either 5 mL of ATCC® Trypsin-EDTA solution or ATCC® Dulbecco's PBS (for more trypsin-sensitive cells) and remove.
4. Add 2 to 3 mL of ATCC® Trypsin-EDTA solution and incubate at the appropriate temperature (4°C, room temperature, or 37°C). Continually check the progress of cell dissociation by microscopy. To avoid clumping, do not agitate the cells by hitting or shaking the flask while waiting for them to detach.
5. Once the cells appear to be detached (5 to 15 minutes for most cell lines, they will appear rounded under the microscope), add 6 to 8 mL of complete growth medium to the cell suspension and with a pipette wash any remaining cells from the bottom of the flask. Check the cells with the microscope to be sure that most (> 95%) exist as single cells. If cell clusters are apparent, continue to disperse the cells with gentle pipetting (see Troubleshooting, below).
6. Add 12 to 15 mL of fresh cell culture media to a new flask and equilibrate this media to the appropriate pH and temperature. Collect the cell suspension, count and/or divide it, and dispense the cells into the newly prepared flask. Refer to the cell line product sheet for recommended subcultivation ratios.
7. For serum free or low serum media, remove the ATCC® Trypsin-EDTA solution by gentle centrifugation (5 minutes at 125 x g) and resuspend the cells in fresh medium.

## Troubleshooting

### Cells are difficult to remove.

- The dissociating agent is too weak. Try incubating at higher temperatures.
- Inhibitors in the medium (e.g., serum) are inactivating the trypsin. Rinse the cell monolayer more thoroughly before incubating with ATCC® Trypsin-EDTA solution.
- Cells have been at confluent density for a too long and the cell-to-cell junctions are so tight that they are preventing the enzyme from reaching the substrate-cell interface. Subculture cells before they are 100% confluent.

### Cells clump after dissociation.

- DNA has been released from lysed cells because the dissociation procedure was too harsh. Add a drop of sterile DNase (1 mg/ml in water) to the cell suspension. In the future, treat the cells more gently during pipetting, shorten the incubation period, and/or decrease the incubation temperature.
- Cells are reaggregating before subculturing. Hold the cell suspension on ice if there will be a delay between removing cells from the flask and dispersing them into fresh cell culture medium.

### Cells have difficulty reattaching.

- The dissociating enzymes may have stripped necessary attachment proteins from the cell surface. Treat the cells more gently, use less ATCC® Trypsin-EDTA solution, shorten the incubation time, and/or lower the incubation temperature.
- Not enough serum or attachment factors are in the medium (common with serum-free medium). Add attachment factors or use protein-coated plates (collagen, polylysine, gelatin, etc.).
- ATCC® Trypsin-EDTA solution was not inactivated by the cell culture medium (e.g., the serum). Add specific enzyme inhibitors or remove the ATCC® Trypsin-EDTA solution by gentle centrifugation (5 minutes at 125 x g) followed by a medium change.

## Quality Control Specifications

Lot specific results are available in the Certificate of Analysis, which is available at [www.atcc.org](http://www.atcc.org) or by contacting ATCC technical service.

## SPECIFICATION CERTIFICATE

Test	Specification
pH	7.0 to 8.0
Osmolality	283 to 313 mOsm/kg

American Type Culture Collection  
PO Box 1549  
Manassas, VA 20108 USA  
[www.atcc.org](http://www.atcc.org)

800.638.6597 or 703.365.2700  
Fax: 703.365.2750  
Email: [Tech@atcc.org](mailto:Tech@atcc.org)

Or contact your local distributor



Product Sheet

## Trypsin-EDTA Solution, 1X (ATCC® 30-2101™)


Sterility Testing	Pass
Cell Culture Tests – MRC-5 (CCL-171)	Pass
Cell Culture Tests – Vero (CCL-81)	Pass
Porcine Parvovirus (PPV)	Not Detected
Mycoplasma	Not Detected

\*Please consult the Certificate of Analysis for lot-specific test results.

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### ATCC Warranty

The viability of ATCC® products is warranted for 30 days from the date of shipment, and is valid only if the product is stored and cultured according to the information included on this product information sheet. ATCC lists the media formulation that has been found to be effective for this strain. While other, unspecified media may also produce satisfactory results, a change in media or the absence of an additive from the ATCC recommended media may affect recovery, growth and/or function of this strain. If an alternative medium formulation is used, the ATCC warranty for viability is no longer valid.

### Disclaimers

This product is intended for laboratory research purposes only. It is not intended for use in humans.

While ATCC uses reasonable efforts to include accurate and up-to-date information on this product sheet, ATCC makes no warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. ATCC does not warrant that such information has been confirmed to be accurate.

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Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at [www.atcc.org](http://www.atcc.org)

Additional information on this culture is available on the ATCC web site at [www.atcc.org](http://www.atcc.org).

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Manassas, VA 20108 USA  
[www.atcc.org](http://www.atcc.org)

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Fax: 703.365.2750  
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