



# Trypsin-EDTA Solution, 1X

## 30-2101™

### 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

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### Storage Conditions

**Product format:** Frozen

**Storage conditions:** -20°C or colder

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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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### Biosafety Information

ATCC determined that a biosafety level is not applicable to this 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 complete your own risk

assessment and understand any potential hazards associated with the material per your organization's policies and procedures and any other applicable regulations as enforced by your local or national agencies.

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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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## Handling Procedures

**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<sup>®</sup> 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<sup>®</sup> 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<sup>®</sup> Trypsin-EDTA solution or ATCC<sup>®</sup> Dulbecco's PBS (for more trypsin-sensitive cells) and remove.
4. Add 2 to 3 mL of ATCC<sup>®</sup> 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 × g) followed by a medium change.

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## Quality Control Specifications

**Mycoplasma contamination:** Not detected

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## Notes

Each cell line responds to ATCC® Trypsin-EDTA solution in a unique manner. For optimum results, observe the cells during the dissociation process to avoid over-trypsinization. For more information, please refer to the General Protocol for Using ATCC® Trypsin-EDTA Solution. For cell line-specific information, please refer to the product sheet supplied with the cell line, or

contact ATCC® Technical Service. ATCC® Trypsin-EDTA solution is suitable for most but not for all adherent cell lines.

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## Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: Trypsin-EDTA Solution, 1X (ATCC 30-2101)

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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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