

## 0.25% Trypsin Solution without EDTA

### Introduction

This solution contains 0.25% trypsin without EDTA, with a pH range of 7.2-7.8. It is sterile-filtered and ready for direct use in the digestion of cultured cells or certain tissues. This trypsin solution offers a convenient and rapid digestion process, typically detaching most adherent cells within approximately 1 min at room temperature.

### Storage

Store at 4°C for 1 year. For long-term storage, store at -20°C.

### Protocol

#### 1. Digestion of adherent cells

- 1) Remove the culture medium and rinse the cells once with sterile PBS, Hanks' Balanced Salt Solution (HBSS), or serum-free medium.
- 2) Add a small volume of 0.25% Trypsin Solution (without EDTA), just enough to cover the cell. Incubate at room temperature for 30 sec to 2 min. Digestion times may vary depending on the cell type.
- 3) Monitor digestion under a microscope. When cells appear shrunken and rounded, or detach easily upon gentle pipetting, immediately add complete culture medium containing serum to terminate the reaction. Then gently pipette to dislodge the cells for subsequent experiments.
- 4) If digestion is insufficient, add fresh trypsin solution and continue digesting. If the digestion time is too long and cells begin to detach spontaneously from the vessel surface, immediately harvest all cells by pipetting directly within the trypsin solution. Centrifuge at 1000-2000 × g for 1 min. Discard the supernatant and resuspend the cell pellet in fresh complete medium containing serum for subsequent experiments.

#### 2. Digestion of tissues

- 1) Digestion times vary significantly depending on tissue type. Terminate digestion once the tissue can be fully dissociated into a single-cell suspension.

## ■ Note

1. Avoid microbial contamination during use.
2. Avoid over-digestion, as prolonged exposure can impair cell viability.
3. Do not warm the whole bottle to room temperature or 37°C. Repeated warming cycles will reduce enzymatic activity. If pre-warming is required, only thaw or warm the specific volume needed for the experiment.
4. For your safety and health, please wear lab coats and gloves during the experiment.
5. For research use only. Not to be used in clinical diagnostic or clinical trials.



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