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Cell passage protocol for TR146 human buccal carcinoma cell line

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We use this protocol and it's working

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Abstract

This protocol outlines the maintenance, subculturing, and cryopreservation of TR146 oral squamous carcinoma cells for use in cancer research

Materials

- DMEM/F12 Medium with 10% FBS
- Trypsin-EDTA (0.25%)
- DMSO (20% in Medium)
- PBS (Phosphate-Buffered Saline)
- 15 mL Conical Tubes
- Tissue Culture Flasks (T75, T50, T25)
- Transfer Pipettes
- Centrifuge
- 37°C Water Bath
- Personal Protective Equipment (PPE)

Safety warnings

Ensure a sterile environment to prevent cell contamination

Subculturing TR146 Cells

10m

1 **Aspirate spent media** using sterile vacuum system

2 **Wash monolayer** with 5 mL PBS at room temperature

3 **Add 3 to 5 mL trypsin** (37°C pre-warmed)

4 **Incubate immediately for 5 min** at 37°C (5% CO_2)

5m

5 **Neutralize** with 5 mL complete DMEM-F12

6 **Centrifuge suspension:** 300×g

5m

7 **Remove supernatants** via suctioning

8 **Resuspend pellet** in 3 mL fresh medium

Seeding Densities

9

	Vessel	Cell Suspension	Medium Volume
	T75	400 µL	10 mL
	T25	200 µL	5 mL

Recommended seeding densities

Cryopreservation

10 **Resuspend pellet** from step 6 in 1.5 mL medium

11 **Add 1.5 mL 20% DMSO-medium** (keep ice-cold)

12 **Aliquot** 1 mL/vial (3 vials per T75)

13 **Freeze** at -80°C (overnight) → LN2 storage

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