

**GP2D Cells | 305778**

**Description**

GP2d is a human colorectal adenocarcinoma cell line derived from a poorly differentiated colon tumor. It was established alongside a sister line, GPSd, from the same adenocarcinoma specimen. While both lines share similar genetic alterations consistent with common patterns seen in colorectal cancer, including an inverted duplication involving chromosome 10q11-q21, they differ markedly in their phenotypic characteristics and cellular behavior. Notably, no translocations involving the ret proto-oncogene-mapped to this chromosomal region-were detected by Southern blot analysis, suggesting the duplication did not disrupt this gene directly.

GP2d cells exhibit a cohesive, spreading growth pattern from the edges of microcolonies to form a confluent epithelial monolayer. This morphology is accompanied by distinct expression patterns of adhesion molecules such as  $\alpha$ 2-integrin, desmoplakin, and E-cadherin, all of which play roles in maintaining epithelial integrity. Functionally, GP2d cells respond robustly to epidermal growth factor (EGF), transforming growth factor-alpha (TGF $\alpha$ ), and insulin, as demonstrated by increased cell proliferation in response to these ligands. Interestingly, both GP2d and GPSd express comparable numbers of EGF receptors, but differ in their expression of EGF receptor ligands. GP2d cells have abundant amphiregulin mRNA, whereas GPSd predominantly expresses TGF $\alpha$  mRNA with little to no amphiregulin, correlating with the differing biological responses observed.

These features make GP2d a valuable model for studying the regulation of growth factor signaling and cell adhesion in colorectal cancer. Its responsiveness to EGF pathway stimuli and distinct epithelial morphology highlight its utility in investigating tumor cell differentiation and proliferation. Furthermore, the shared origin with GPSd allows for comparative studies of clonal variation within tumors, particularly in the context of ligand-receptor dynamics and epithelial-to-mesenchymal transition (EMT) responses.

**Organism** Human

**Tissue** Colon

**Disease** Adenocarcinoma

**Synonyms** Gp2d, Gp2D, GP2D

**Age** 71 years

**Gender** Female

**Ethnicity** Caucasian

**Growth properties** Adherent

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<b>Citation</b>	GP2D (Cytion catalog number 305778)
<b>Biosafety level</b>	1
<b>NCBI_TaxID</b>	9606
<b>CellosaurusAccession</b>	CVCL_2450
<b>Mutational profile</b>	Mutation: KRAS, Simple, p.Gly12Asp (c.35G>A), Heterozygous, TP53
<b>Culture Medium</b>	DMEM, w: 4.5 g/L Glucose, w: 4 mM L-Glutamine, w: 3.7 g/L NaHCO <sub>3</sub> , w: 1.0 mM Sodium pyruvate (Cytion article number 820300a)
<b>Supplements</b>	Supplement the medium with 10% FBS
<b>Dissociation Reagent</b>	Accutase
<b>Subculturing</b>	Remove the old medium from the adherent cells and wash them with PBS that lacks calcium and magnesium. For T25 flasks, use 3-5 ml of PBS, and for T75 flasks, use 5-10 ml. Then, cover the cells completely with Accutase, using 1-2 ml for T25 flasks and 2.5 ml for T75 flasks. Let the cells incubate at room temperature for 8-10 minutes to detach them. After incubation, gently mix the cells with 10 ml of medium to resuspend them, then centrifuge at 300xg for 3 minutes. Discard the supernatant, resuspend the cells in fresh medium, and transfer them into new flasks that already contain fresh medium.
<b>Freeze medium</b>	As a cryopreservation medium, we use complete growth medium (including FBS) + 10% DMSO for adequate post-thaw viability, or CM-1 (Cytion catalog number 800100), which includes optimized osmoprotectants and metabolic stabilizers to enhance recovery and reduce cryo-induced stress.

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### Thawing and Culturing Cells

1. Confirm that the vial remains deeply frozen upon delivery, as cells are shipped on dry ice to maintain optimal temperatures during transit.
2. Upon receipt, either store the cryovial immediately at temperatures below  $-150^{\circ}\text{C}$  to ensure the preservation of cellular integrity, or proceed to step 3 if immediate culturing is required.
3. For immediate culturing, swiftly thaw the vial by immersing it in a  $37^{\circ}\text{C}$  water bath with clean water and an antimicrobial agent, agitating gently for 40-60 seconds until a small ice clump remains.
4. Perform all subsequent steps under sterile conditions in a flow hood, disinfecting the cryovial with 70% ethanol before opening.
5. Carefully open the disinfected vial and transfer the cell suspension into a 15 ml centrifuge tube containing 8 ml of room-temperature culture medium, mixing gently.
6. Centrifuge the mixture at  $300 \times g$  for 3 minutes to separate the cells and carefully discard the supernatant containing residual freezing medium.
7. Gently resuspend the cell pellet in 10 ml of fresh culture medium. For adherent cells, divide the suspension between two T25 culture flasks; for suspension cultures, transfer all the medium into one T25 flask to promote effective cell interaction and growth.
8. Adhere to established subculture protocols for continued growth and maintenance of the cell line, ensuring reliable experimental outcomes.

### Incubation Atmosphere

$37^{\circ}\text{C}$ , 5%  $\text{CO}_2$ , humidified atmosphere.

### Shipping Conditions

Cryopreserved cell lines are shipped on dry ice in validated, insulated packaging with sufficient refrigerant to maintain approximately  $-78^{\circ}\text{C}$  throughout transit. On receipt, inspect the container immediately and transfer vials without delay to appropriate storage.

### Storage Conditions

For long-term preservation, place vials in vapor-phase liquid nitrogen at about  $-150$  to  $-196^{\circ}\text{C}$ . Storage at  $-80^{\circ}\text{C}$  is acceptable only as a short interim step before transfer to liquid nitrogen.

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**Sterility**

Mycoplasma contamination is excluded using both PCR-based assays and luminescence-based mycoplasma detection methods.

To ensure there is no bacterial, fungal, or yeast contamination, cell cultures are subjected to daily visual inspections.