

Modulation of Pancreatic Cancer Cell Activity in CO₂-Free Culture Using TES and MOPS Buffers

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Overview

Pancreatic ductal adenocarcinoma (PDAC):

- Accounts for >90% of all pancreatic cancers
- Among the most lethal malignancies worldwide

Cell model:

- PANC-1 cells (human PDAC-derived) widely used for in vitro studies

Culture challenge:

- Standard conditions: 37 °C, 5% CO₂ → bicarbonate buffering maintains physiological pH
- Without CO₂, culture pH becomes unstable → assays give unreliable results

Previous findings (UCLan):

- MTT (viability) and LDH (cytotoxicity) outcomes adversely affected in CO₂-free culture

Study aim:

- Test whether Good's buffers (TES, MOPS) can stabilize pH, improve assay consistency, and enhance reliability in CO₂-independent conditions

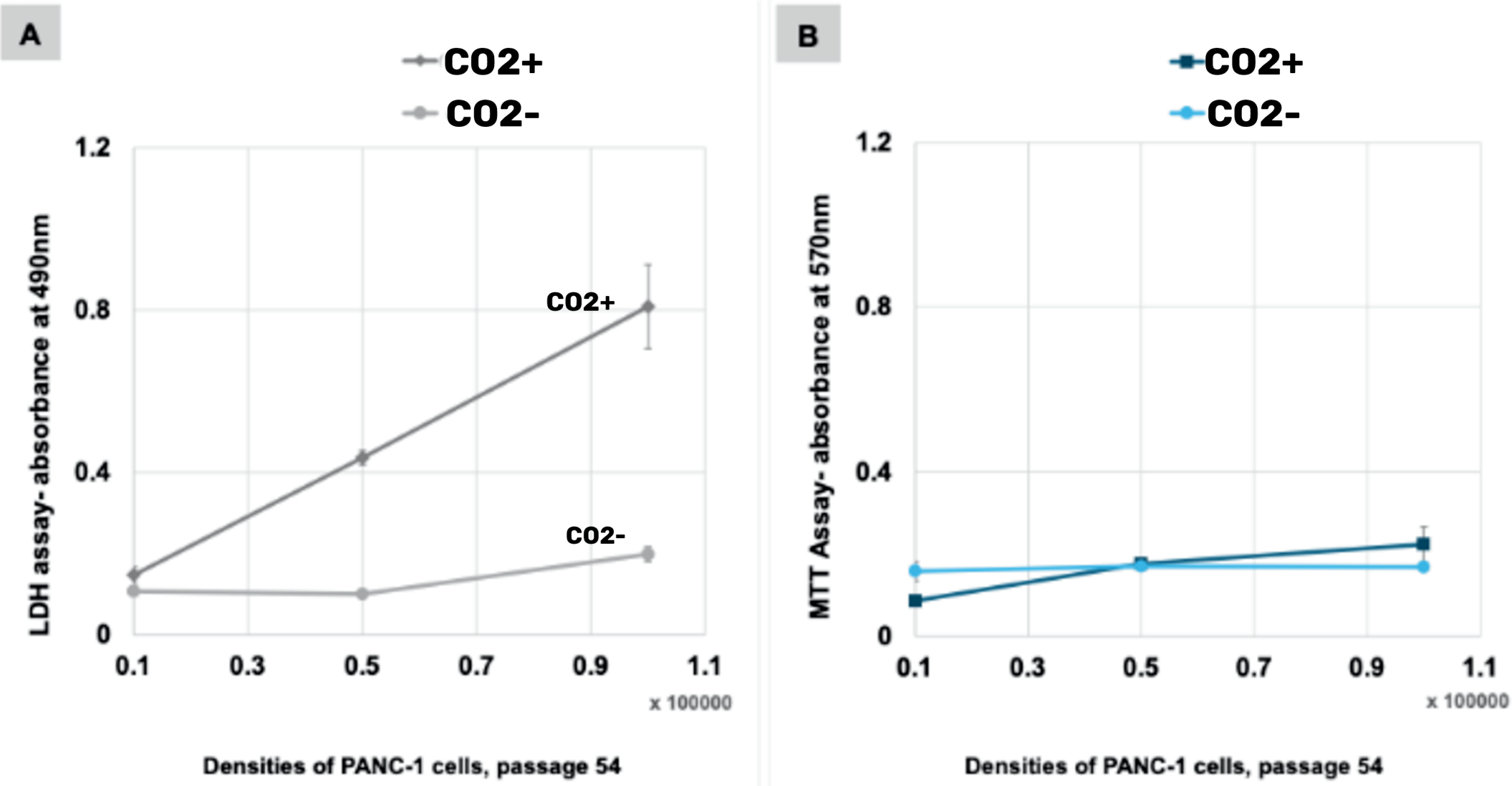
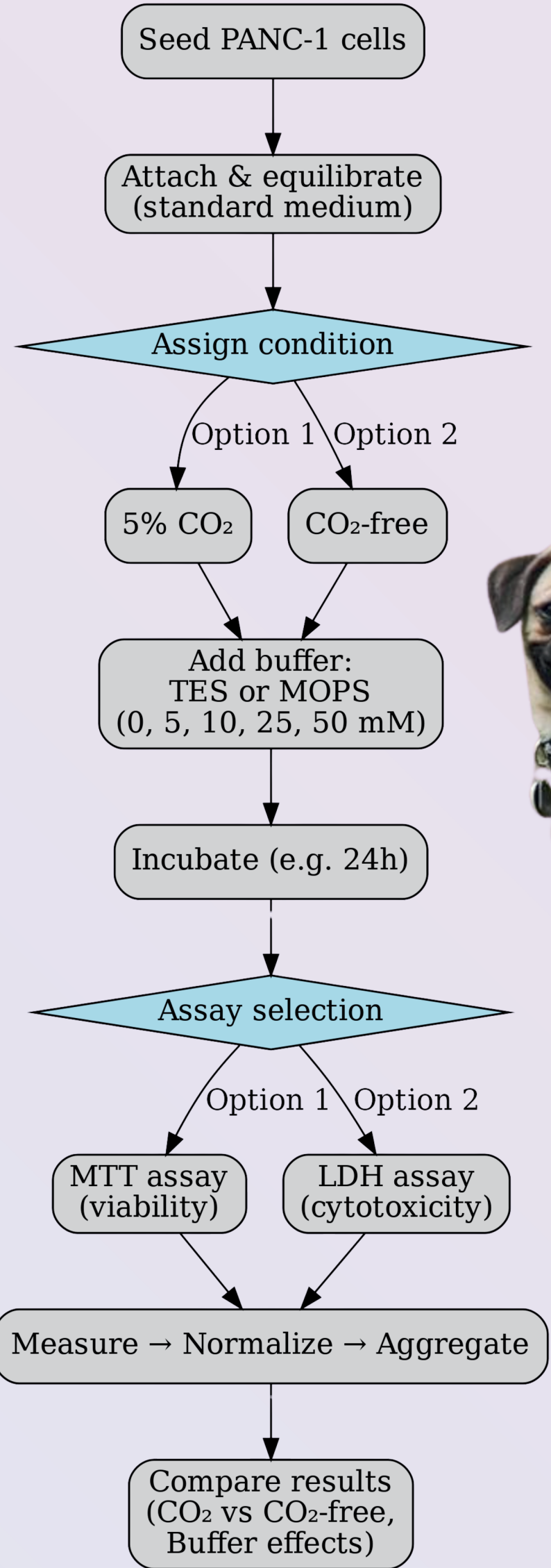
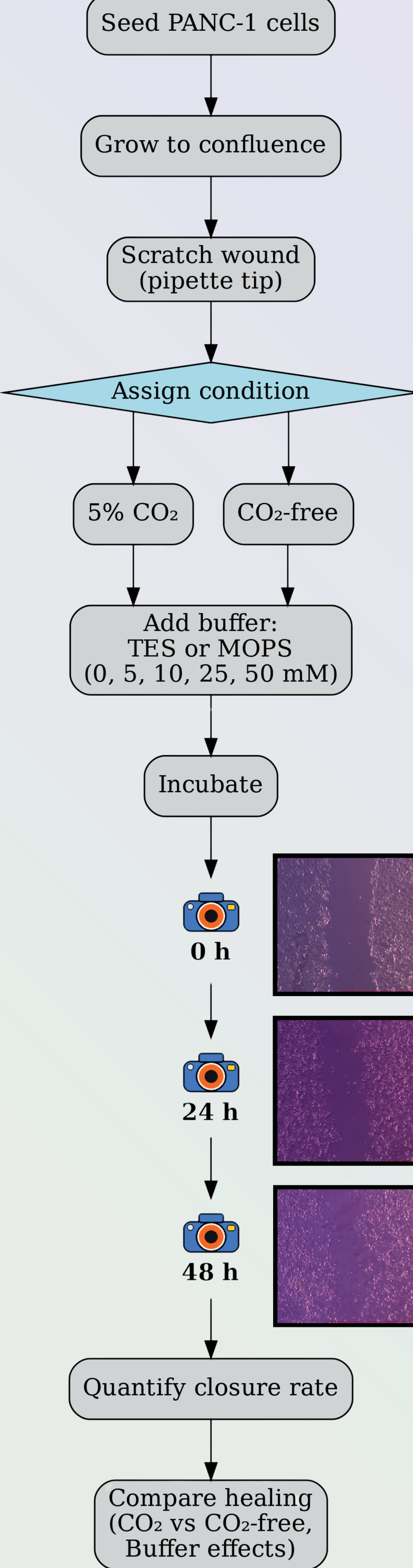


Figure 1. Comparison between CO₂+/- conditions using MTT and LDH assay (prelim experiment) Suggesting that CO₂-free culture conditions may alter MTT and LDH assay outcomes compared with standard 5% CO₂ culture. This raises the question of whether observed effects reflect true biological changes or artifacts introduced by the culture environment. Before these assays can be used reliably, it is necessary to determine the extent to which CO₂ itself contributes to the results. Taken from Nusrat Mouni, 2024, unpublished.

Experiment Workflow Phase I



Experiment Workflow Phase II



Results Phase I

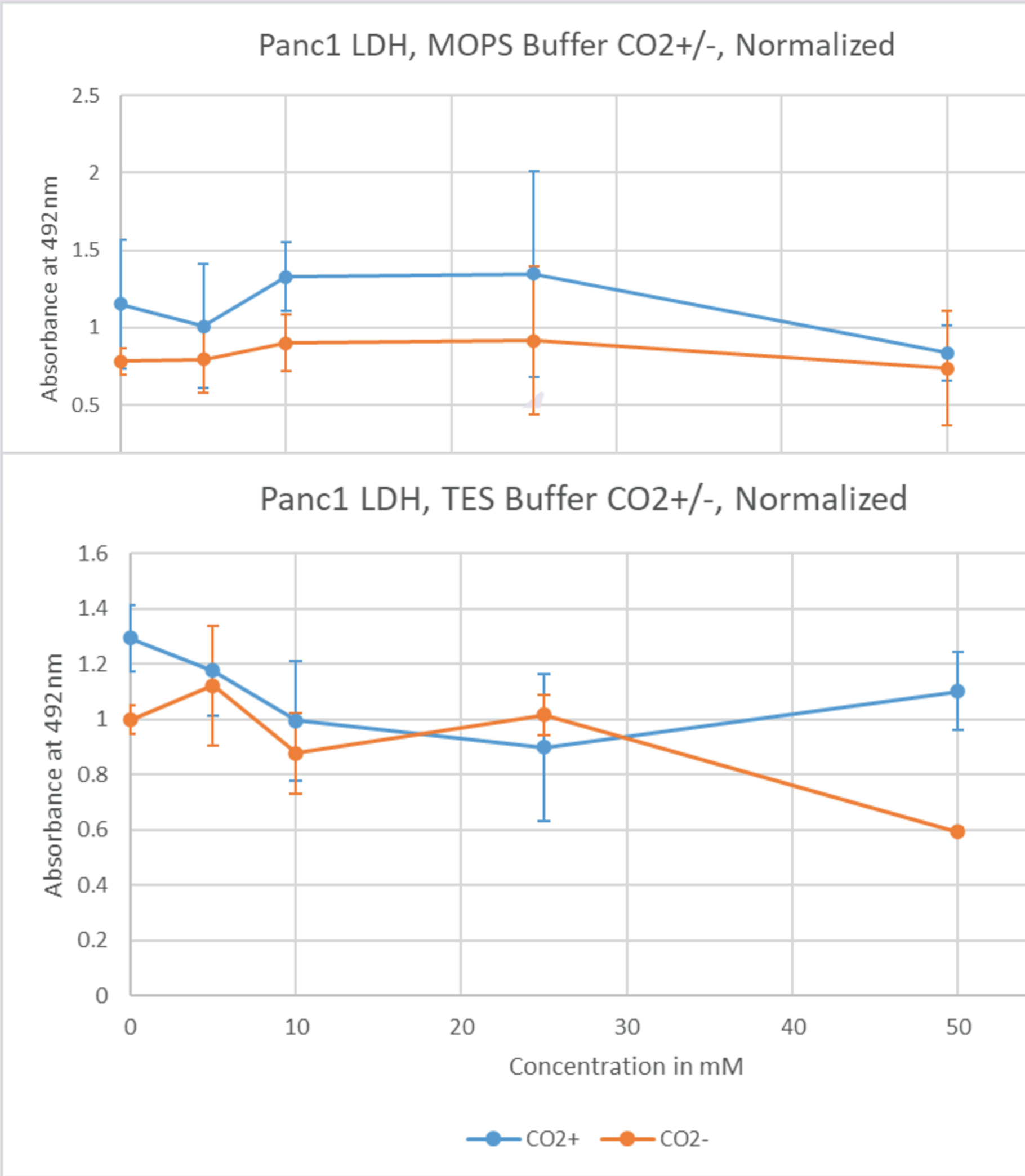


Figure 2. Effects of Good's buffers on PANC-1 LDH assay.

Normalized results for MOPS and TES buffer treatments are shown. No consistent trend was observed across concentrations or conditions. Before normalization, control groups displayed high variability and poor reproducibility, with unpredictable distributions.

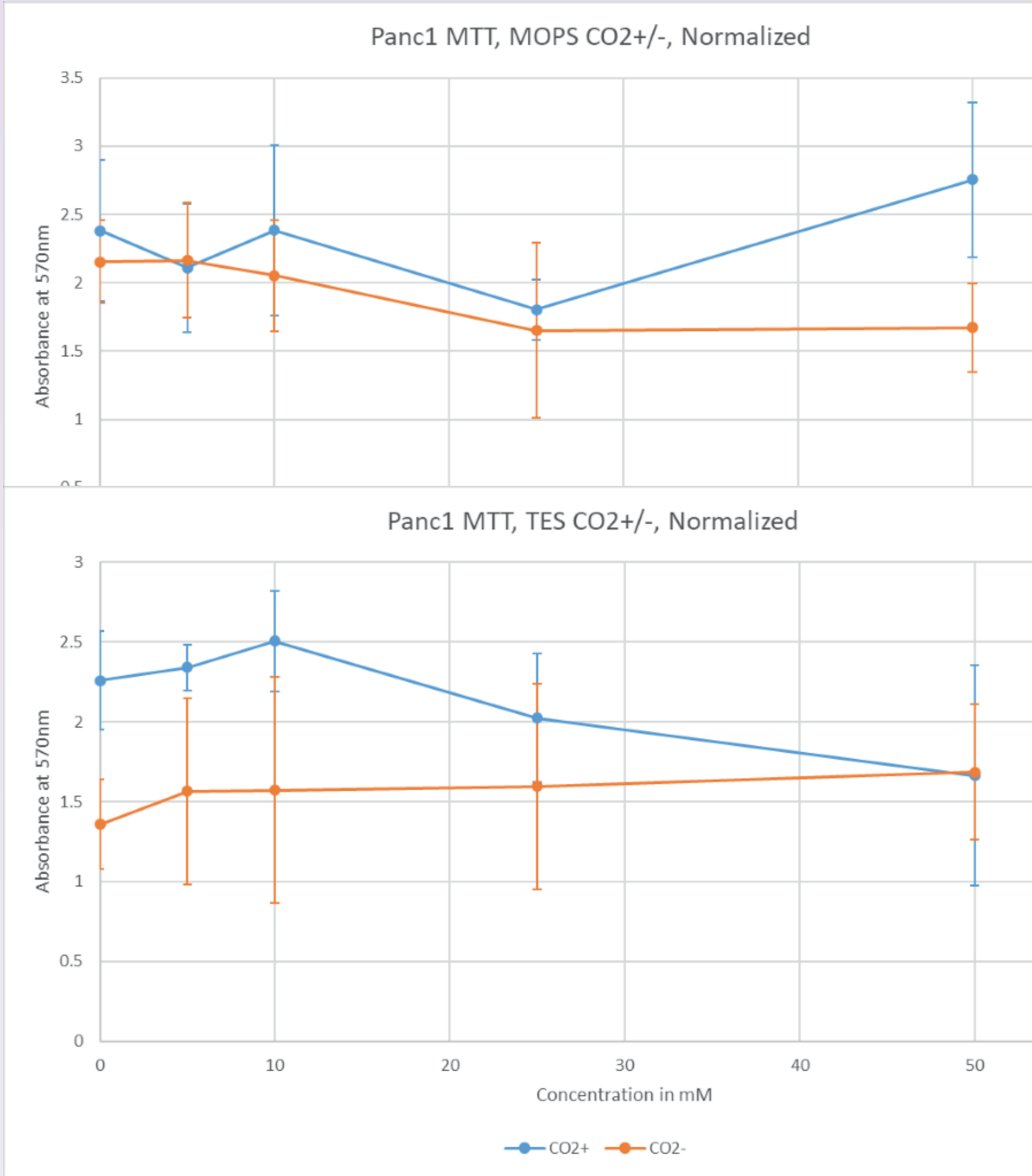
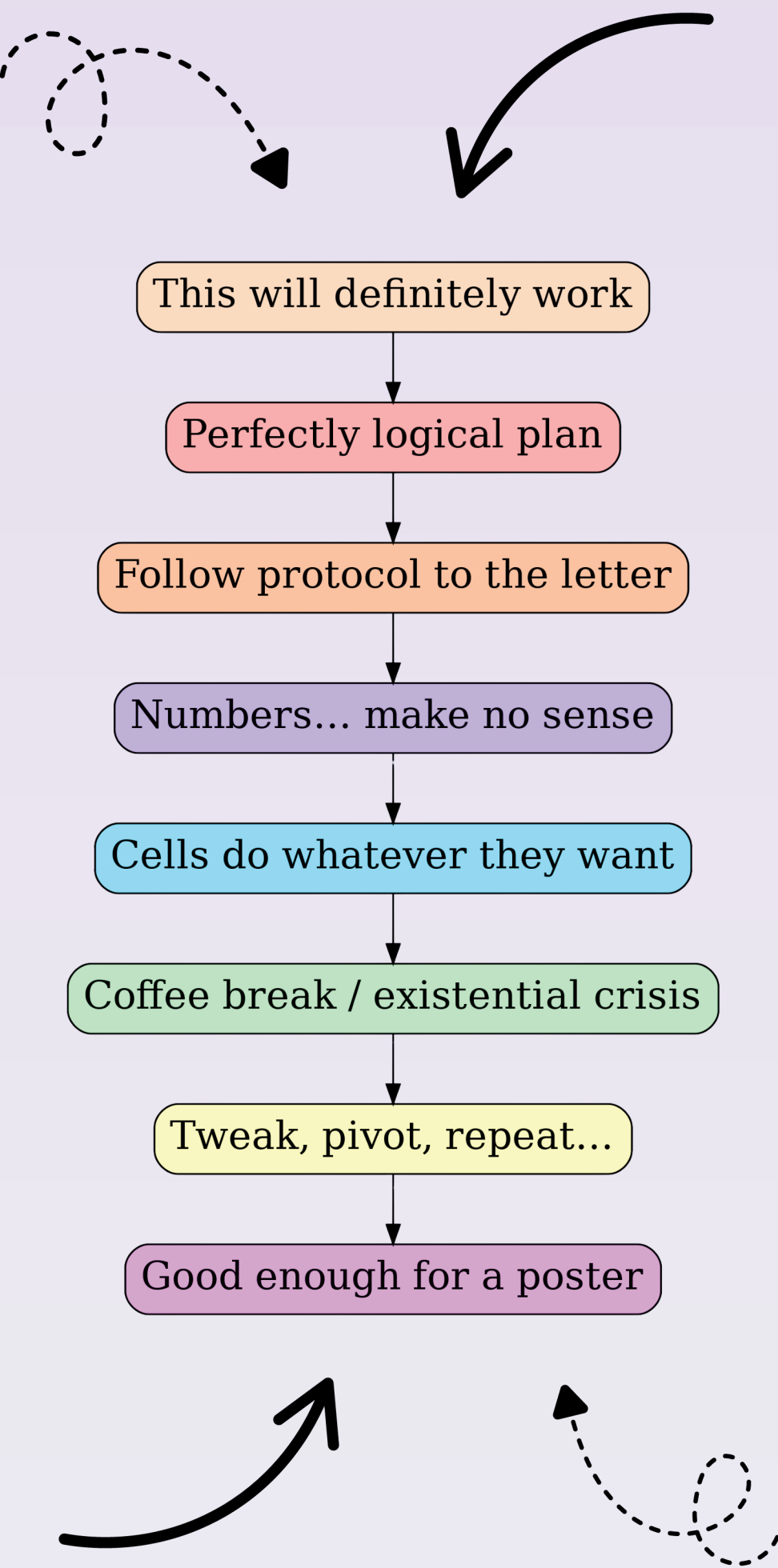


Figure 3. Effects of MOPS and TES buffers on PANC-1 MTT assay.

This figure combines results from both buffer treatments under CO₂-free and standard CO₂ culture conditions. No consistent advantage was observed for either condition, as trends varied across experiments. Control groups also fluctuated substantially, limiting reproducibility.

Results Phase II

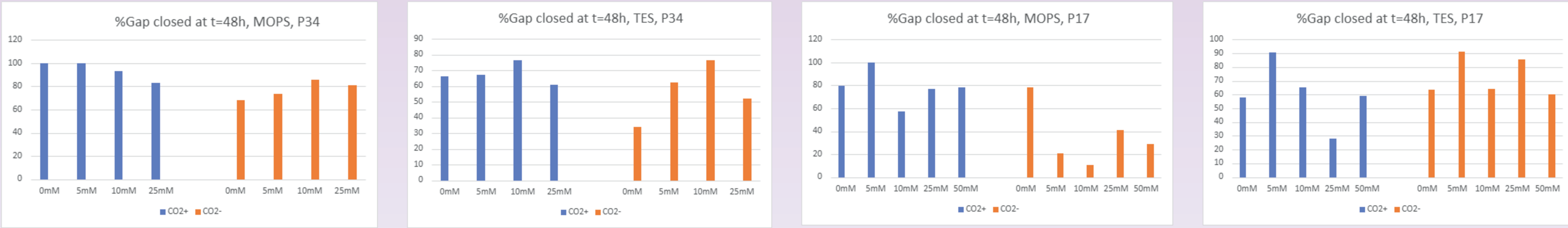


Figure 4. Percentage wound closure at 48 h in PANC-1 scratch assays under TES or MOPS treatment.

Representative bar graphs show the proportion of the wound gap closed after 48 h, quantified from images using ImageJ. Each graph corresponds to a different treatment condition (TES or MOPS, at indicated concentrations) or passage number.

Discussion

In Phase I, both TES and MOPS buffers were found to introduce substantial variability into the assays. This volatility extended beyond treated samples, as even control groups occasionally produced inconsistent results without any intervention. While frustrating, these findings are valuable they emphasize the need for caution when interpreting MTT or LDH assays, particularly in co-culture systems with bacterial infection. This is especially true when the LDH assay is applied post-lysis, where results may be further confounded.

In Phase II, the dataset was limited (two biological repeats, no technical repeats), and thus no firm conclusions can be drawn. Preliminary trends suggest that passage number may influence outcomes: at higher passages, a gap appeared between CO₂ and CO₂-free controls, which could be partially equilibrated by buffer supplementation, until higher concentrations introduced toxicity. At lower passages, however, this baseline gap was absent, and buffer addition instead led to erratic behaviour with no consistent pattern.

Taken together, the results highlight both the challenges and the nuances of working with PANC-1 cells under altered buffering systems. If there is a single take-home message from Phase I, it is this: be wary of quick interpretations, for these cells are clearly mischievous.

