

Serum-free dilution to render TrypLE Express ineffective

Introduction

Standard dissociation reagents such as trypsin often require the presence of specific reagents or serum to reduce their activity after dissociation; however, some workflows may not allow for the addition of inhibitors to the resuspended, dissociated cells. One advantage of Gibco™ TrypLE™ Express enzyme is that it can be rendered ineffective by dilution alone, without the need for inhibitory reagents or serum.

Demonstrating the minimum serum-free dilution for rendering TrypLE Express enzyme ineffective

To verify the effectiveness of dilution alone in rendering TrypLE Express enzyme ineffective, we measured the confluency of a monolayer of QM7 (quail myoblast) cells before and after 5 minutes of 37°C incubation with equivalent volumes of varied dilutions of TrypLE Express enzyme and serum-free basal medium. Figure 1 demonstrates that at minimum, a 1:1 dilution of TrypLE Express enzyme to serum-free basal medium was able to completely prevent dissociation of the monolayer for 5 minutes of incubation at 37°C, whereas the undiluted enzyme under the same condition allowed for complete dissociation.

To further demonstrate the effects of dilution alone, we plated dissociated QM7 cells in various dilutions of TrypLE Express enzyme in serum-free basal medium, for 1 hour at 37°C, and then exchanged the medium in the wells with complete medium. After 24 hours, the confluency of the cells was measured. Figure 2 shows that culture in a 1:1 mixture of TrypLE Express enzyme and serum-free basal medium for 1 hour at 37°C allowed for cell attachment that was not significantly different from culture in a 1:9 dilution.

Overall, the results demonstrate that TrypLE Express enzyme can be rendered ineffective by dilution alone, without the need for serum or specific inhibitors, with dilutions as low as 1:1. To achieve optimal cell health after dissociation, it is still recommended to dilute higher if possible with the appropriate medium and/or use centrifugation to remove TrypLE Express enzyme.

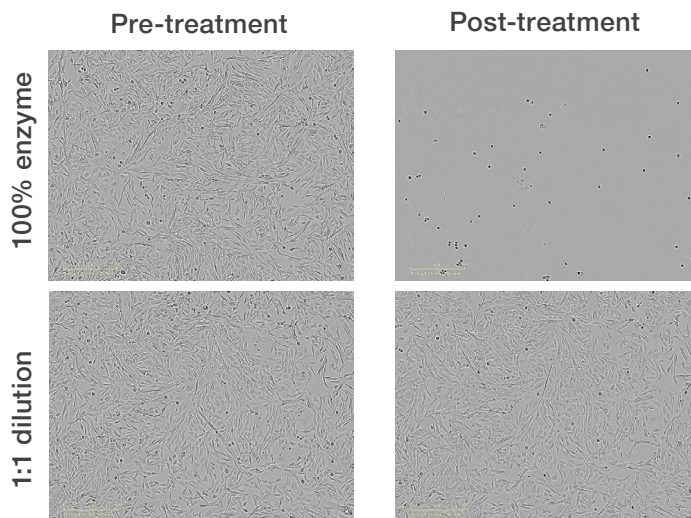
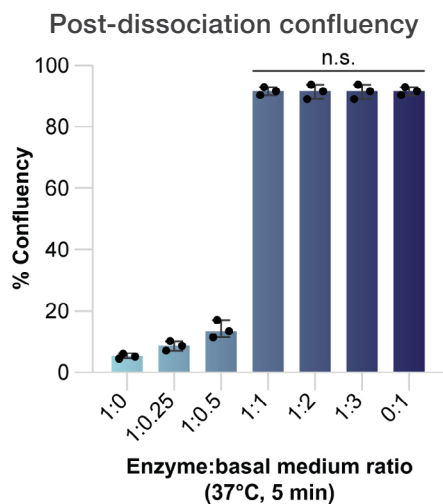


Figure 1. Dilution of TrypLE Express enzyme to prevent dissociation. Bar graph of average confluency measurements (n = 3 wells) and representative phase-contrast images of QM7 cells before and after 5 minutes of incubation at 37°C with dilutions of TrypLE Express enzyme in serum-free basal medium. All conditions under the “n.s.” line of the bar graph are not significantly different by one-way ANOVA multiple comparisons.

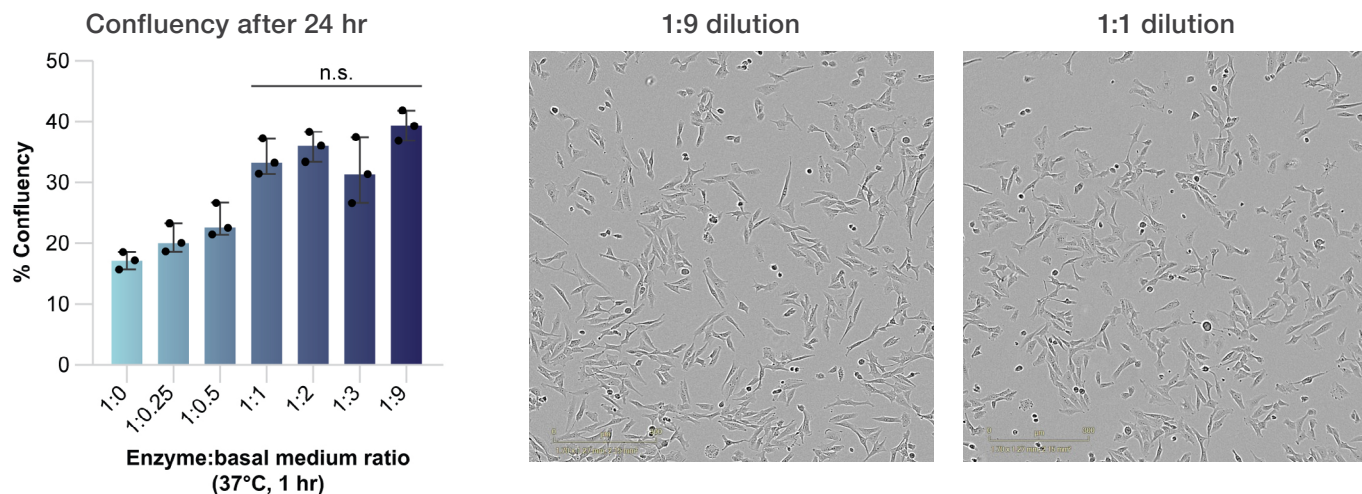


Figure 2. Dilution of TrypLE Express enzyme to allow for cell adherence. Bar graph of average confluency measurements (n = 3 wells) and representative phase-contrast images of QM7 cells in complete medium for 24 hours after 1 hour of post-plating incubation at 37°C with dilutions of TrypLE Express enzyme in serum-free basal medium. All conditions under the “n.s.” line of the bar graph are not significantly different by one-way ANOVA multiple comparisons.

Product	Quantity	Cat. No.
TrypLE Express Enzyme (1X), no phenol red	20 x 100 mL	12604039
Trypsin-EDTA (0.25%), phenol red	500 mL	25200072
Trypsin Neutralizer Solution	100 mL	R002100
DMEM, high glucose	500 mL	11965092
PBS, pH 7.4 (no calcium, no magnesium)	500 mL	10010023
Fetal Bovine Serum, Premium	500 mL	A5670701
Nunc Cell-Culture Treated 6-Well Multidish, Nunclon Delta Surface	75/case	140675

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