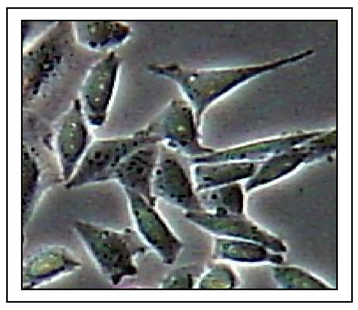

# Efficacy and Toxicity of Three Commercially Available Recombinant Trypsins versus Porcine Trypsin in Three Different Cell Lines

*Kyle Liu, Hans Huttinga, and John F Menton* - **Kerry,** 3400 Millington Road, Beloit, WI 53511
john.menton@kerry.com - http://www.SheffieldBioScience.com

## Introduction:

Trypsin is a serine protease found in the digestive system of most mammals. In cell culture, trypsin derived from porcine pancreases has historically been utilized. Trypsin is used to detach cells that grow in adherent monolayers on culture flasks. It is also used in primary cell culture/ cell isolation techniques to break down clumps of tissue into singular cells. Trypsin also has an application in influenza vaccine production in MDCK cell lines, where it increases virus infectivity by cleaving haemagluttinin. Although porcine trypsin is utilized for a variety of cell culture applications, the trend toward animal component free (ACF) media ingredients has led to an increasing interest in recombinant trypsin (rTrypsin). In this study, the performance of three commercially available rTrypsins was assessed alongside a native trypsin (animal derived) in 3 different cell lines. The cell detachment efficacy and the cell toxicity of the three commercially available rTrypsins were compared as well.

## Materials and methods:

Kerry's Sheffield™ rTrypsin is available in lyophilized form and can be readily solubilized in a buffer containing EDTA and salts. Recombinant Trypsins from competitors were purchased directly from the manufacturer. Native porcine trypsin was purchased from SIGMA. Trypsin enzyme activity (BAEE units) was measured using Trypsin Activity Assay Kit (Abcam) to normalize the enzyme units. Cell images were taken using a Leica inverted light microscope at 100x. The cell density and viability were counted with a NovaBiomedical Analyzer.

MRC-5, MDCK and VERO cells were cultivated in 10 % FBS supplemented DMEM in triplicate in 25 $cm^2$ flasks. When the cell density reached > 90 % confluence, each cell line was detached with a different trypsin. The time for monolayer detachment was recorded at various time points and the action of each trypsin was filmed. To see if any cumulative toxicity occurred after treatment with rTrypsin, the population doubling length (PDL) of each cell line was calculated after 5 passages with each trypsin. To compare the cell detachment efficacy between different commercially available rTrypsins, the same enzyme unit was utilized for each rTrypsin in the experiments.

## Results and Conclusion:

It was found that all the trypsin products were efficient at detaching monolayers in all cell types indicating the animal component free trypsins can replace the native trypsin for cell detachment (Figure 1). However, none of the trypsin evaluated were capable of completely removing the tight intracellular junctions formed by these cells. In terms of time required to remove the monolayer, Sheffield™ rTrypsin from Kerry proved to be the fastest enzyme when compared to other commercially available rTrypsins (Fig. 2A&B). The cell toxicity study showed that the cell viability and population doubling length were similar in all the commercially available rTrypsins demonstrating the recombinant trypsin doesn't cause obvious toxicity to the cells (Fig. 3&4).

The study shows, in a broad range of cell lines, that recombinant trypsin is a viable alternative to native trypsin in terms of effectiveness and risk mitigation. Sheffield™ rTrypsin is as efficient as other commercially available rTrypsins for the detachment of vaccine cell lines.

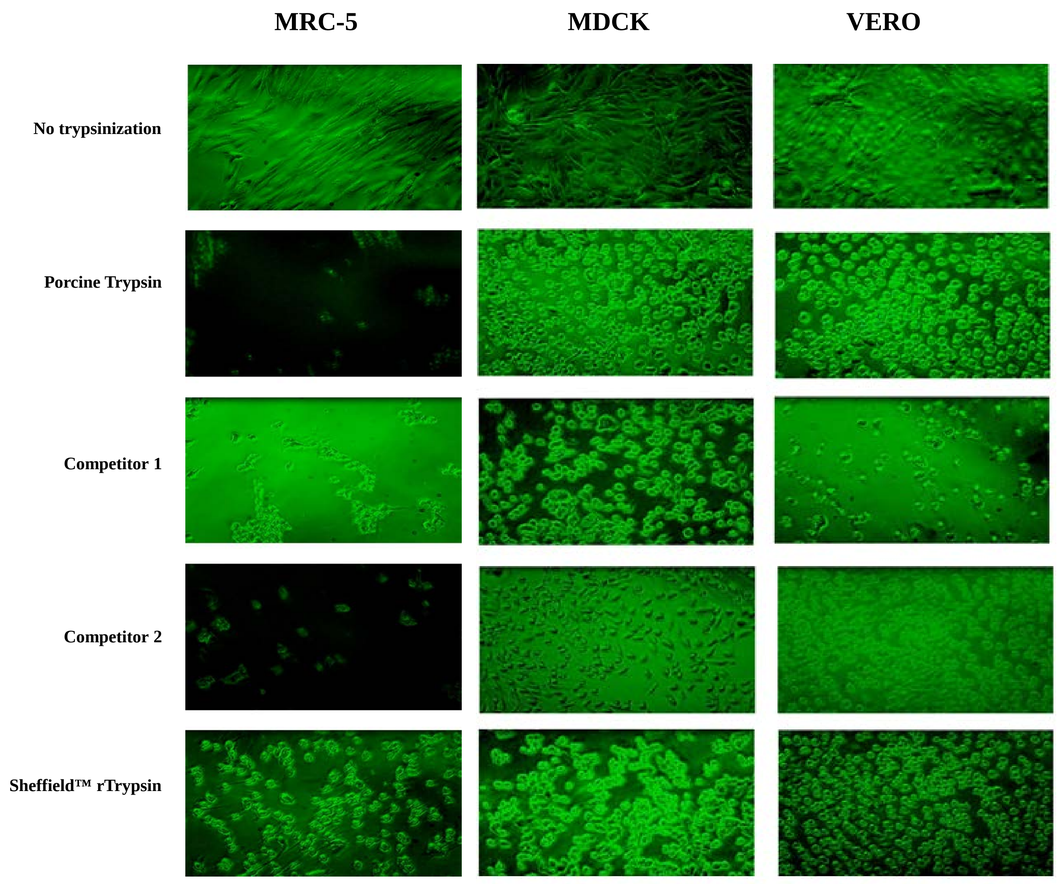


**Fig. 1. Animal component free rTrypsins have the same efficacy for cell detachment as native Trypsin.**

Recombinant rTrypsins showed the same efficacy for cell dissociation as the native trypsin. None of the trypsins evaluated were capable of completely removing the tight intracellular junctions formed by these cells

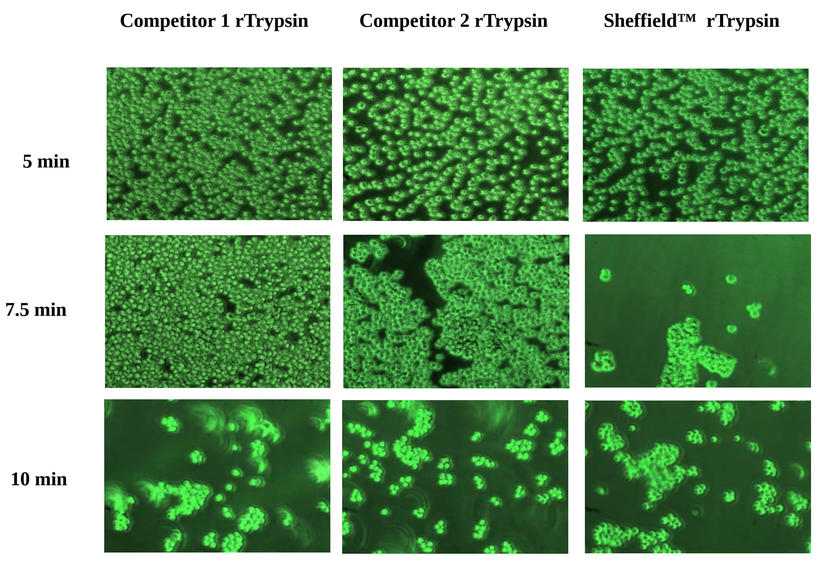


**Fig. 2A. Cell morphology showed Sheffield™ rTrypsin detached cells faster than other commercially available rTrypsins**

Vero cells (grown to 90-100 % confluence) were exposed to competitor 1, competitor 2 and Sheffield™ rTrypsin at 37 ˚C at various time points. Sheffield™ rTrypsin showed fast cell dissociation compared to other commercially available rTrypsins although all the trypsins used the same amount of enzyme unit.

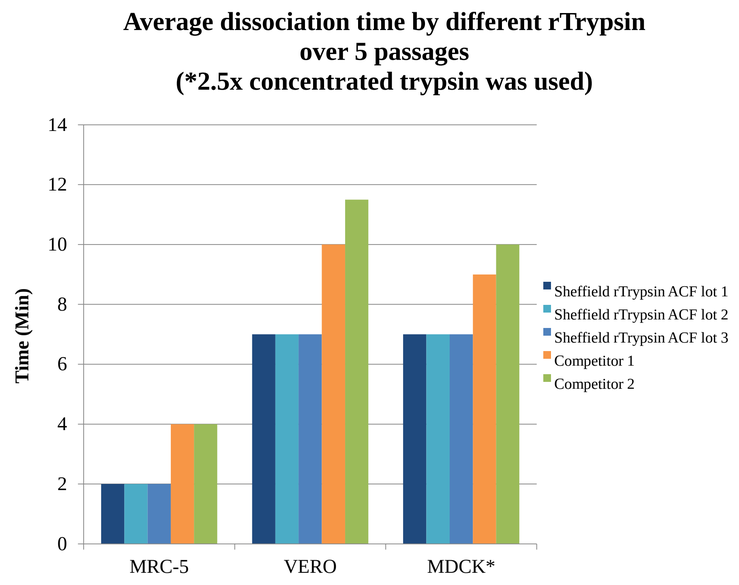


**Fig. 2B. Dissociation time showed Sheffield rTrypsin detached cells faster than other commercially available rTrypsins**

Cells were dissociated by each rTrypsin solution at the same enzyme unit, respectively. After 5 time sequential experiments, the average dissociation time was calculated. The shorter dissociation time was observed in Sheffield™ rTrypsin solution.

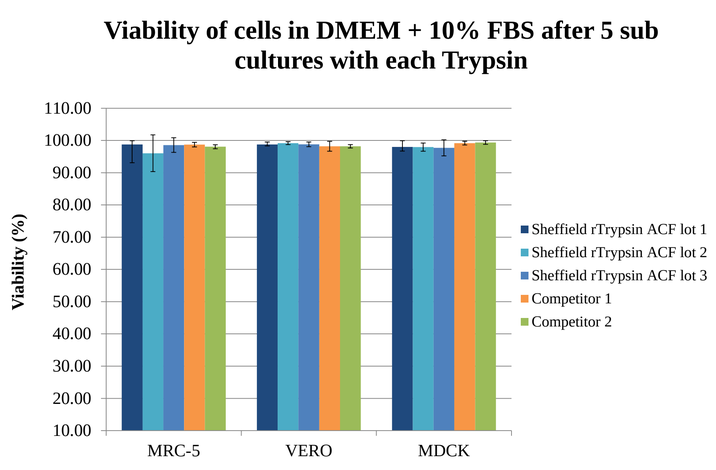


**Fig. 3. No obvious toxicity was found in Sheffield™ rTrypsin and other commercially available rTrypsins.**

Attached cells were dissociated with each rTrypsins and cell viabilities were measured. As is shown, viability of cells dissociated by Sheffield rTrypsin was equivalent to the viability of cells dissociated by other rTrypsins.

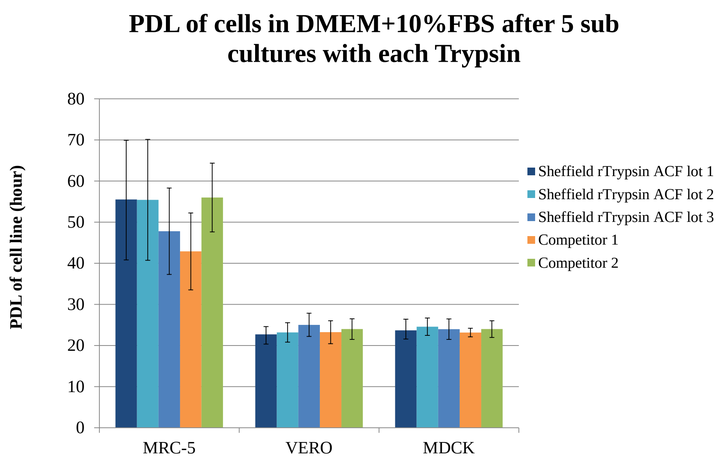


**Fig. 4. Population doubling levels confirm no toxicity.**

MRC-5, MDCK and VERO cells were cultured in DMEM +10 % FBS after dissociation with each Trypsin solution, respectively, average population doubling time was calculated after 5 time sequential passages. No obvious toxicity was observed for any one of the rTrypsins.