Performance Comparison of the Sarstedt Cell Scraper

SARSTEDT AG & Co. KG



Background

During routine cell culture it is necessary to harvest cultured cells from the growth surface for continued culture (splitting) and for other terminal applications. Although more routine, enzymatic treatments are incompatible with many cell lines and downstream procedures, necessitating the use of mechanical lifting to remove cells. Because the amount of harvested material is the limiting factor in many biochemical assays and screening processes there is a serious need for laboratories to identify the most efficient and effective tools for mechanical cell harvesting.

Due to the variety of materials and blade designs available, it is difficult for any laboratory to select a cell scraper type without first performing testing. For busy labs this is not practical, especially for a device like a cell scraper, which is an often overlooked and underappreciated piece of technology. The current study was conducted in order to objectively evaluate the performance of cell scrapers from several leading cell culture ware manufacturers by performing a harvesting experiment.

Method

- NIH-3T3 cells were cultured in DMEM/low glucose (supplemented with 10 % FCS, 2 mM L-Glutamine) on 35 mm TC dishes (Sarstedt).
 Cells were cultured under standard conditions (37°C, 5 % CO₂, humidified atmosphere) until >90 % confluency was achieved.
- Cells were removed using the identical scraping method: 6-8 strokes across middle of dish followed by at least two full revolutions around the side-wall. If possible, the blade was oriented perpendicular to the handle to allow a more productive "rake" motion of harvesting

- (Sarstedt and competitors B and D). Scrapers without this option (A and C) were used in the manufacturer-recommended side-to-side, "sweeping" motion. Scraping force, stroke number, and scraping mechanics were kept as uniform as possible with a manual process such as this. Trypsin control was performed according to standard trypsinization protocol.²
- Cells were spun at 200 x g for 8 minutes. Supernatant was gently aspirated by manual pipette so as not to disrupt the pellet. 0.5 ml trypsin was added, and the pellet manually triturated. After ~10 minutes in trypsin, 0.5 ml of culture medium was added and the sample triturated into a single cell suspension. Cell counting was performed on a Countess automated cell counter (Invitrogen) according to manufacturer's instructions. Plates were stained using crystal violet according to standard protocol. ²

Results

NIH-3TC cells were cultured until > 90 % confluent (Fig. 1).



Fig. 1: Light microscopic image of NIH-3T3 cells prior to cell harvesting.

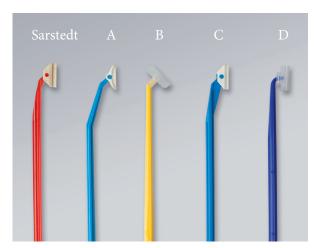
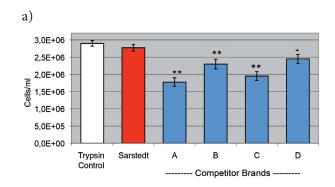
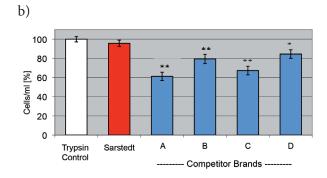


Fig. 2: Cell scrapers used for comparative analysis: Sarstedt, Competitors A, B, C, D (left to right).

At least cells from four confluent 35 mm dishes were scraped with cell scrapers from Sarstedt and four competitor products (Fig. 2). The number of cells obtained were compared to cell numbers obtained by standard trypsinization protocol.

As shown in figure 3, trypsin control revealed almost 3 x 10^6 cells per 35 mm dish. Cell counts obtained with the competitor scrapers were significantly lower than the trypsin control (p = 0.001 - 0.01), while Sarstedt scrapers gave counts that were statistically identical to the trypsin control (p = 0.08) (Fig. 3c). When normalized to the trypsin control, 96 % of cells could be removed with the Sarstedt cell scraper, whereas cell losses between 16% - 39% could be detected with competitor cell scrapers (Fig. 3b).





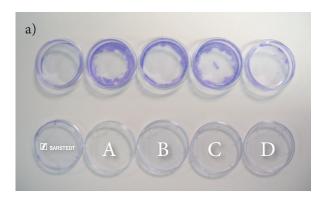
Manufacturer	Sarstedt	Α	В	C	D
p value	0.080	0.001	0.001	0.002	0.014

Fig. 3 Cell Scraping performance

c)

- a) Total cell counts obtained with cell scrapers from Sarstedt and four competitors.
- b) Cell counts normalized to 100 % trypsin control.
- c) Significance for all analysis was set at ** p < 0.01, * p < 0.05 using student's t-test.

Cell numbers obtained were confirmed by staining the residing cells on the 35 mm dishes with crystal violet. Figure 4 shows representative dishes illustrating the inferior harvest using competitor scrapers. The violet staining clearly shows that especially in the boundary area of the dishes cells could not efficiently be scraped off the surface.



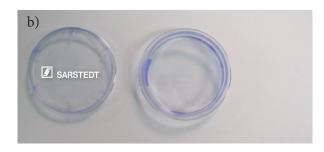


Fig. 4
Crystal violet staining of 35 mm dishes after cell scraping
a) residual violet stained cells on the dishes scraped with a Sarstedt
cell scraper or with competitor products.
b) Magnification of the dish scraped with a Sarstedt cell Scraper.

Conclusion

Although considered a commodity in most labs, care should be taken when selecting a cell scraper as varying brands do not afford comparable performance. As shown here, mechanical cell harvesting efficiency, effectiveness, and consistency can be greatly impacted by the cell scraper brand employed. To maximize cell culture yields and cell viability while minimizing waste, the results obtained support the use of superior quality Sarstedt cell scrapers.

References

- 1. Huang, et. al. Trypsin-induced proteome alteration during cell subculture in mammalian cells. Journal of Biomedical Science 2010, 17:36.
- 2. Culture of Animal Cells: A Manual of Basic Technique/R. Ian Freshney.-6th ed. p. cm. Chapter 15: Characterization, (15.5.2), 249-250.

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