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Eppendorf Cell Culture Consumables – Improved optical performance facilitates microscopic analysis of cells

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Abstract

Microscopic analysis of cells using an inverted microscope is a daily routine procedure in every cell culture laboratory. Visual examination of cultures is crucial e.g. to assess the degree of confluence of the cell layer and confirm the absence of contaminants. Furthermore a lot of cell culture based assays performed in plate formats are analyzed using manual or automated microscopic techniques. Besides instrumentation, the choice of the consumable strongly influences the microscopic results: e.g. meniscus formation of the liquid results in optical interference disturbing the phase contrast effect. Cells can hardly be focused with sufficient contrast, thus making it difficult to use this technique in smaller formats like 96-well plates. Moreover interfering shadows may limit the area of observation and a poor surface planarity may result in increased refocusing. The precise design with enhanced planarity and the minimized meniscus formation in Eppendorf Cell Culture Consumables result in a uniform illumination, a minimum of refocusing and an excellent phase contrast, thus facilitating a quick and precise microscopic analysis of cells.

Introduction

The daily analysis of cell cultures using an inverted microscope is an essential step in cell handling. From simple routine procedures – like the quick visual check of cell morphology or the assessment of confluence of the cell layer – to complex analysis of cell based assays, a quick and reliable optical examination is crucial for successful cell culture research. The following factors are considered as a prerequisite for efficient microscopic analysis: a minimum of refocusing across the area of observation, a uniform illumination without interfering shadows and a high-contrast image of the cells across the whole growth area. Besides the microscope itself, the consumable strongly influences the operational procedure and the imaging results in different ways:

Focus stability:
The material, bottom thickness and geometry influence the planarity of a cell culture consumable. Glass for instance is a very rigid and inflexible material that is highly planar and very well suited for high resolution imaging. Polystyrene, the material cell culture consumables are made of, is more flexible resulting in a less planar surface in comparison to glass. A consumable with a poor bottom planarity may result in increased refocusing and can make manual microscopic analysis a time-consuming and cumbersome procedure. Furthermore the repetitive motion of the hand constantly moving the focus knob to readjust the microscope objective to the right focus level means a less ergonomic work situation. This can be tedious especially when analyzing a large amount of vessels on a daily basis or many well to well analyses in plate formats have to be performed. Using an automated microscope e.g. for high content analysis (HCA) of cells solves the ergonomic problem but the laser-based autofocus mechanism implies repeated refocusing of the lens resulting in prolonged analysis steps or even worse, in less reproducible results.

Meniscus formation and phase contrast performance:
Phase contrast microscopy is the most common contrast enhancing light microscopic technique that is used for cell analysis as it creates high-contrast images of usually transparent specimens such as living cells. A common problem in phase contrast microscopy is the meniscus formation at the air-liquid interface. The curvature of the liquid causes a refraction of the light with the result that cells can hardly be displayed with a satisfactory contrast thus making it difficult to use this technique in smaller formats, like 96-well plates. There are specialized consumables available for meniscus correction [1] but using standard cell culture formats the meniscus is something that can hardly be avoided. In addition to its optical disadvantage – the loss of image quality – the meniscus is also associated with a phenomenon that is called the halo cell growth effect: cells often tend to adhere as a halo in dishes or plates in relation to the center. It has been suggested that the meniscus formation of the media leads to this irregular cell growth when seeding cells or even re-feeding cells with new media [2].

Illumination of the imaging area:
Interfering shadows at the well edges are a common problem when working with multiwell plates limiting the area of observation. This can be a disadvantage especially in applications, where a uniform illumination of the whole growth area is crucial e.g. for single cell cloning experiments: here interfering shadows at the well edges make it more difficult or even impossible to identify cell clones growing at the periphery of the well.

In this Application Note we compare the optical performance of Eppendorf Cell Culture Consumables with corresponding products from other suppliers: We analyzed planarity, focus stability, phase contrast image quality and shadow formation.

Material and Methods

Planarity and focus stability:
Whole plate planarity of different 96-well polystyrene cell culture plates was measured by optical contact scanning (n=4). To analyze focus stability across the growth area NIH 3T3 cells were seeded at equal density in 100 mm Eppendorf Cell Culture Dishes, and comparable dishes from other suppliers (competitor A, B). Cells were analyzed 48 hours post seeding with an inverted microscope using a 10 x objective (Axio Observer A1, Zeiss®). Cells were focused in the center of the dish and then the dish was moved to different points of the growth area without refocusing. Pictures were taken at three different points of the dish (see drawing in figure 2) and it was compared if the cells can be seen in focus.

Phase contrast performance:
To examine the meniscus effect in phase contrast inverted microscopy, CHO-K1 cells were seeded at equal density into 96-well cell culture plates. After 48 hours the plates were analyzed with phase contrast microscopy with 20 x and 40 x microscope objectives (CKX41, Olympus).

Illumination of the imaging area:
Microscopic studies were performed to compare the illumination at the edge of individual wells in 96-well cell culture plates. Therefore adipose-derived mesenchymal stem cells (AdMSCs) were seeded at equal density into 96-well plates. After 5 days of cell growth, cells were analyzed first with phase contrast microscopy (CK40, Olympus) and, following DAPI (nuclei) staining, with fluorescence microscopy (Life Technologies EVOS®FL, Thermo Fisher Scientific).

Results and Discussion

Planarity and focus stability:
Focus stability across the cell growth area is associated with the planarity of the consumable. An enhanced planarity results in less refocusing. As shown in figure 1 the Eppendorf dish displays a clear "in focus" high-contrast picture of the cell layer at all three observation points in the dish. Nearly no refocusing is needed whereas the tested cell culture dishes from other suppliers display a blurred "out of focus" picture of the cells when moving away from the central focus point without a readjustment of the microscope objective. The good focus stability indicates the enhanced bottom planarity of the Eppendorf Cell Culture Consumables.

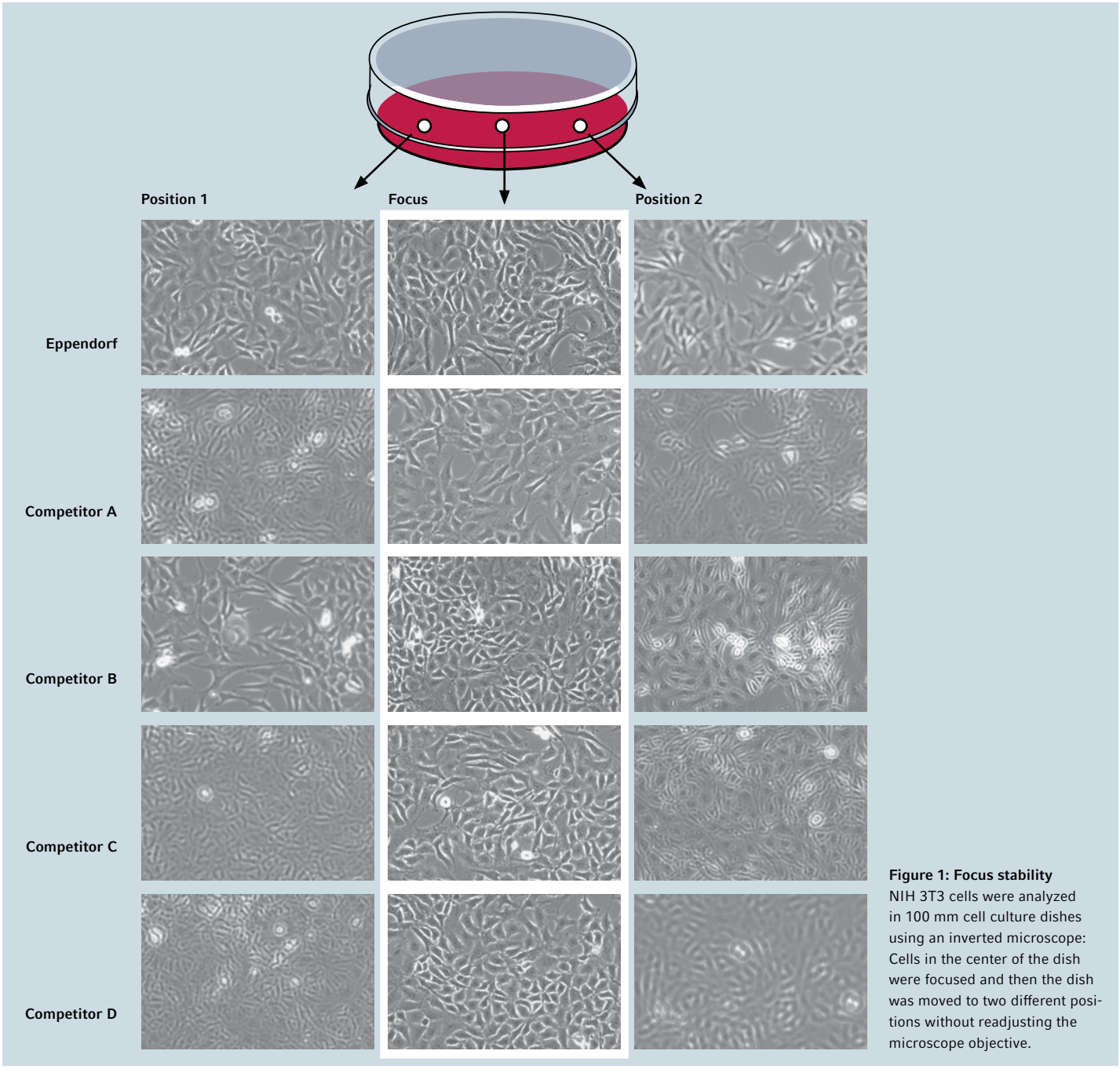
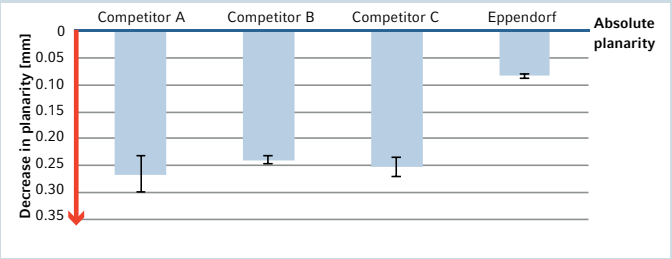


Figure 1: Focus stability
NIH 3T3 cells were analyzed in 100 mm cell culture dishes using an inverted microscope: Cells in the center of the dish were focused and then the dish was moved to two different positions without readjusting the microscope objective.

The optical contact scanning analysis shows that the Eppendorf 96-Well Cell Culture Plate has the best whole plate planarity of all analyzed cell culture plates (see figure 2).

96-well plates: Deviation from absolute planarity



	Competitor A	Competitor B	Competitor C	Eppendorf
Deviation from absolute planarity [mm]	+/- 0.266	+/- 0.240	+/- 0.253	+/- 0.085

*Absolute planarity = 0 mm; value indicates the deviation from absolute planarity

Figure 2: Surface planarity
Different 96-well cell culture plates were measured by optical contact scanning. Displayed are the deviations from maximum whole plate planarity in mm. The Eppendorf Cell Culture Plate, 96-Well shows the highest whole plate planarity.

Meniscus formation and phase contrast performance:
Figure 3 shows that in the Eppendorf Cell Culture Plate, 96-Well the liquid meniscus is reduced to a minimum. The liquid meniscus is a result of the tissue culture treatment of cell culture consumables, which makes the usually hydrophobic polystyrene hydrophilic. For the Eppendorf tissue culture (TC) treated products the standard, well established technique, of a so called corona discharge is used. This process generates charged groups on the usually hydrophobic polystyrene surface. This facilitates cell attachment and spreading of most adherent cells. But unlike most products from other suppliers, a specific technique is used which limits the TC treatment to the well bottom exclusively. Therefore the sides of the walls remain hydrophobic. This leads to the reduced liquid meniscus and improved optical results observed in Eppendorf Cell Culture Consumables.

Minimized meniscus in Eppendorf 96-Well Cell Culture Plate

Typical meniscus formation in 96-well cell culture plate

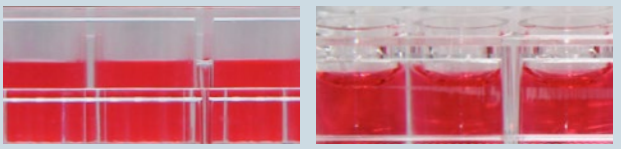


Figure 3: Liquid meniscus in 96-well cell culture plates

To evaluate the effect of the meniscus on the phase contrast image performance, cells were seeded in 96-well cell culture plates and analyzed with phase contrast microscopy using 20 x and 40 x objectives with an inverted microscope.

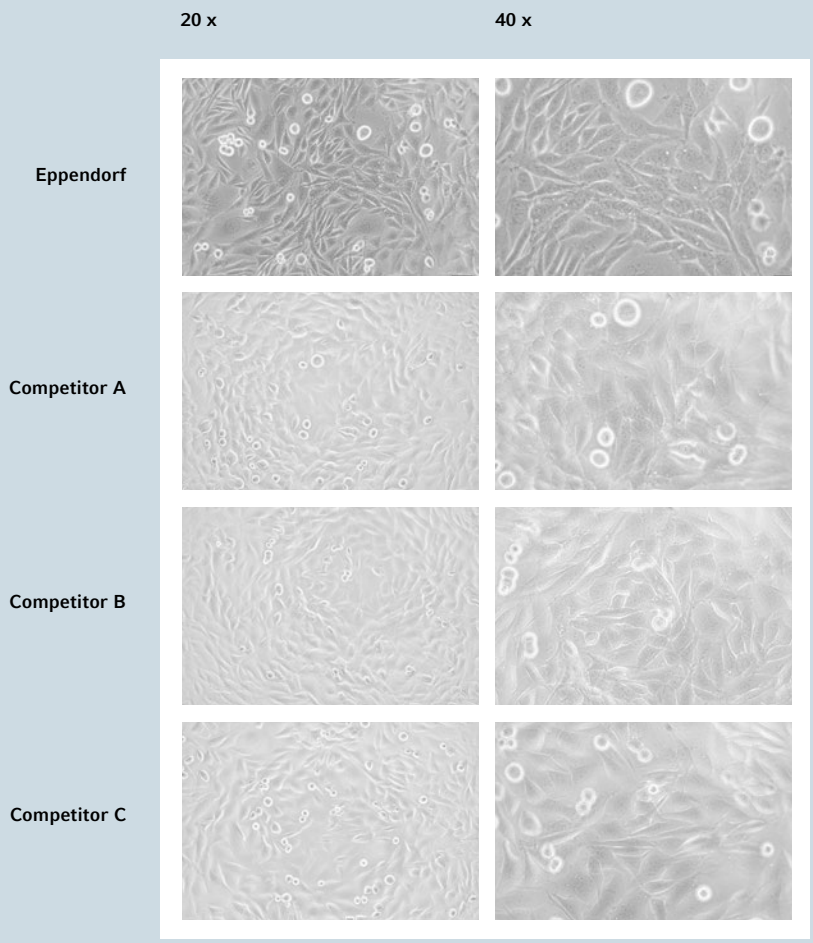


Figure 4: Phase Contrast performance:
To examine the meniscus effect in phase contrast microscopy, CHO-K1 cells were evaluated in 96-well cell culture plates with phase contrast microscopy using 20 x and 40 x objectives.

As shown in figure 4 an improved phase contrast can be achieved within the Eppendorf plate at all magnifications. With both magnifications single cells can be clearly observed with high contrast in the Eppendorf plate in comparison to similar plates from other suppliers where cells appear poor in contrast and hard to focus. The liquid meniscus results in optical interference disturbing the phase contrast effect (see figure 5a). The absence of a liquid meniscus prevents the light refraction leading to an even background illumination and an unaffected image contrast (see figure 5b). The result is an excellent phase contrast throughout the observation area.

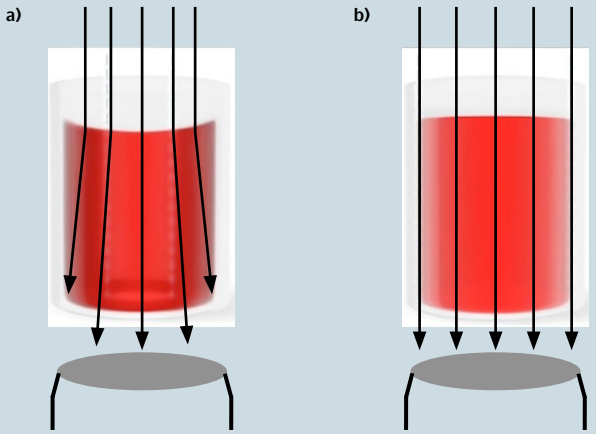


Figure 5: Meniscus formation:
a) The curvature of the liquid meniscus creates light refraction leading to a poor phase contrast resulting in a loss of image quality
b) No scattering of the light occurs resulting in a uniform illumination and a clear phase contrast.

Illumination of the imaging area:
Phase contrast and fluorescence microscopy studies were performed to compare illumination at the edge of individual wells within each 96-well cell culture plate. In the Eppendorf plate the wells show no shadow that interferes with imaging or visual observation. In contrast the wells from both analyzed competitor plates display a black circular shadow limiting the analyzing area and thus interfering with both light and fluorescence microscopy observations (see figure 6). The uniform illumination of the well is associated with the reduced liquid meniscus and the precise well geometry of the Eppendorf Cell Culture Plate.

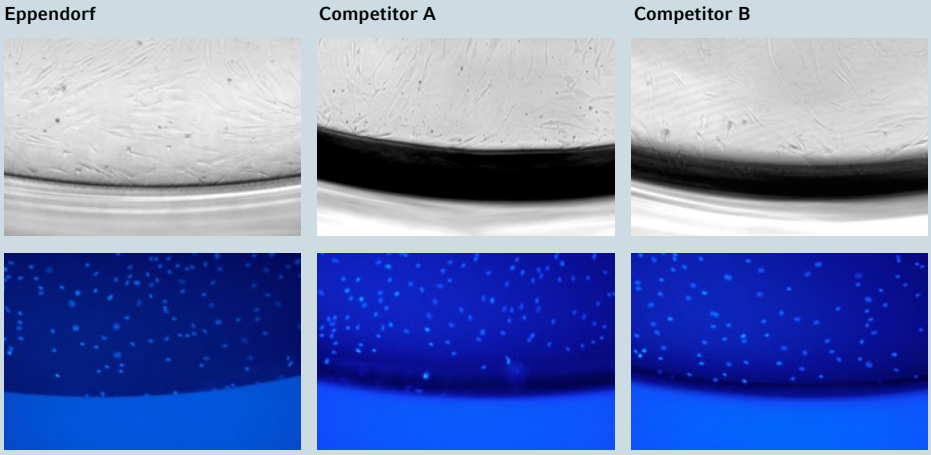


Figure 6: Illumination of the observation area
Microscopy studies were performed to compare the illumination at the edge of individual wells within 96-well cell culture plates. AdMSCs were analyzed in 96-well cell culture plates with phase contrast microscopy (upper panel) and with fluorescence microscopy after DAPI staining (lower panel). An interfering shadow can be observed with the competitors at the edge of the wells whereas with the Eppendorf plate the interfering shadow is reduced to a minimum.

Conclusion

An efficient microscopic analysis is crucial in cell culture research, from basic routine check of cell morphology to reliable cell-based assay analysis. Eppendorf Cell Culture Consumables show an optimized microscopic performance: The excellent bottom planarity results in minimal refocusing facilitating a quick work procedure. The exact well design and the reduced meniscus enable a uniform illumination without disturbing shadows at the edges and an improved phase contrast over the whole observation area. Because of these properties Eppendorf Cell Culture Consumables are best suited for microscopic applications.

Literature

[1] Horn E, Zantl, R. Phase-contrast light microscopy of living cells cultured in small volumes. Microscopy and Analysis. May 2006; 5–7.
[2] Ryan J.A. American biotechnology laboratory. 1989 7(1):8–16

Ordering information

Description	Order no. international	Order no. North America
Eppendorf Cell Culture Dish, 35 mm, sterile, free of detectable pyrogens, RNase & DNase, DNA. Non-cytotoxic.		
TC treated, 300 dishes (30 bags x 10 dishes)	0030 700.112	0030700112
non-treated, 300 dishes (30 bags x 10 dishes)	0030 700.015	0030700015
Eppendorf Cell Culture Dish, 60 mm, sterile, free of detectable pyrogens, RNase & DNase, DNA. Non-cytotoxic.		
TC treated, 300 dishes (30 bags x 10 dishes)	0030 701.119	0030701119
non-treated, 300 dishes (30 bags x 10 dishes)	0030 701.011	0030701011
Eppendorf Cell Culture Dish, 100 mm, sterile, free of detectable pyrogens, RNase & DNase, DNA. Non-cytotoxic.		
TC treated, 300 dishes (30 bags x 10 dishes)	0030 702.115	0030702115
non-treated, 300 dishes (30 bags x 10 dishes)	0030 702.018	0030702018
Eppendorf Cell Culture Plate, 6-Well, with lid, flat bottom, sterile, free of detectable pyrogens, RNase & DNase, DNA. Non-cytotoxic.		
TC treated, 60 plates, individually wrapped	0030 720.113	0030720113
non-treated, 60 plates, individually wrapped	0030 720.016	0030720016
TC treated, 200 plates (20 bags x 10 plates)	0030 720.121	0030720121
Eppendorf Cell Culture Plate, 12-Well, with lid, flat bottom, sterile, free of detectable pyrogens, RNase & DNase, DNA. Non-cytotoxic.		
TC treated, 60 plates, individually wrapped	0030 721.110	0030721110
non-treated, 60 plates, individually wrapped	0030 721.012	0030721012
Eppendorf Cell Culture Plate, 24-Well, with lid, flat bottom, sterile, free of detectable pyrogens, RNase & DNase, DNA. Non-cytotoxic.		
TC treated, 60 plates, individually wrapped	0030 722.116	0030722116
non-treated, 60 plates, individually wrapped	0030 722.019	0030722019
Eppendorf Cell Culture Plate, 48-Well, with lid, flat bottom, sterile, free of detectable pyrogens, RNase & DNase, DNA. Non-cytotoxic.		
TC treated, 60 plates, individually wrapped	0030 723.112	0030723112
non-treated, 60 plates, individually wrapped	0030 723.015	0030723015
Eppendorf Cell Culture Plate, 96-Well, with lid, flat bottom, sterile, free of detectable pyrogens, RNase & DNase, DNA. Non-cytotoxic.		
TC treated, 80 plates, individually wrapped	0030 730.119	0030730119
non-treated, 80 plates, individually wrapped	0030 730.011	0030730011
TC treated, 200 plates (20 bags x 10 plates)	0030 730.127	0030730127

For complete ordering information please visit www.eppendorf.com/ccc

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