forum
Technical Notes and Applications for Laboratory Work
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Content

CELLSTAR® Cell Culture Vessels with Cell-Repellent Surface

1. Key Facts

- Effectively prevents the process of cell attachment
- For suspension culture of semi-adherent and adherent cell lines
- Ideal surface for spheroid formation
- Perfect for the formation of stem cell aggregates
- Non-cytotoxic
- Free of detectable endotoxins
- Free of detectable DNase / RNase and human DNA
- Available as 100 mm cell culture dish, 6 well multiwell plate, 96 well microplate with F- and U-bottom (additional formats upon request)
- Sterile, individually wrapped, easy to open

greiner bio-one

2. Introduction

For most applications in cell culture, the properties of the vessels to be used are optimised to enhance conditions for cell attachment. With platforms made of polystyrene, this is achieved by using a physical method to treat the surface of the vessels. This treatment leads to the incorporation of polar carboxyl and hydroxyl groups to the hydrophobic polystyrene surface resulting in improved and very regular cell attachment. For fastidious cell lines or applications that will stress the cells, protein coated surfaces or advanced non-biological surfaces are applied.

Nevertheless, for some applications a surface that prevents cell attachment is required. These applications include

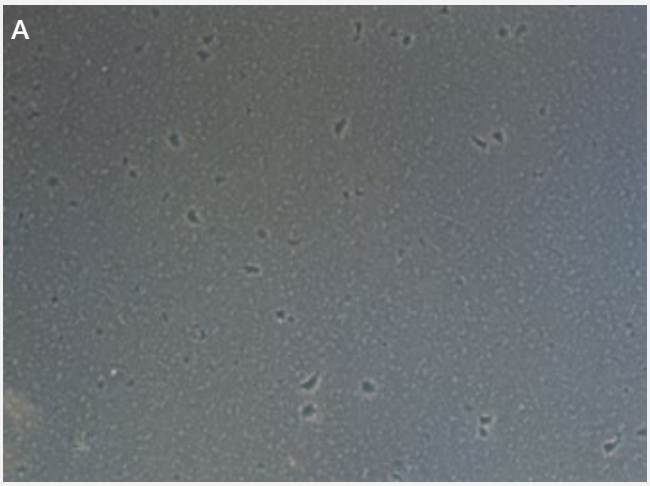
- Spheroid culture
- Aggregation of stem cells
- Suspension culture of semi-adherent and adherent cell lines

The CELLSTAR® cell-repellent surface from Greiner Bio-One fulfils these demands. Achieved through an innovative chemical surface modification, Greiner Bio-One's cell-repellent surface is an ideal substrate for cell culture as it does not degrade or 'leach out' under standard cell culture conditions.

All cell culture vessels with a cell-repellent surface are sterilised by irradiation (SAL of 10^{-3}). They are quality controlled for the absence of detectable endotoxins, DNase/RNase and human DNA and show no cytotoxic effects. Evaluation of cytotoxicity is done in accordance to EN ISO 10993-5 with mammalian cell lines. More detailed information concerning general quality aspects can be found on the Greiner Bio-One website www.gbo.com/bioscience and in our product catalogue.

To control the performance of the surface of cell-repellent cell culture vessels, attachment of cells is compared to the standard tissue culture CELLSTAR® surface. CaCo-2 cells are seeded at a density of 18,000 cells/cm^2 and incubated for 24 hours at 37 °C in an incubator with a 5 % CO_2 environment.

After 24 hours of incubation, the medium is removed, the vessels are washed with PBS and EtOH is added to fix adherent cells, if present. The sample vessels are then examined and compared under a microscope with 10-fold magnification (Fig. 1).



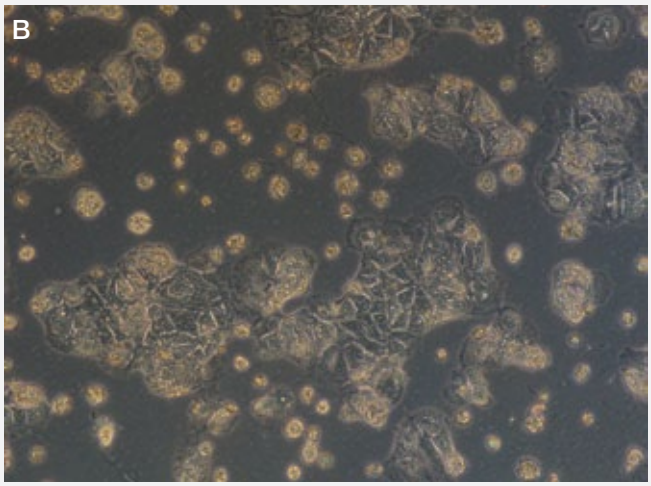
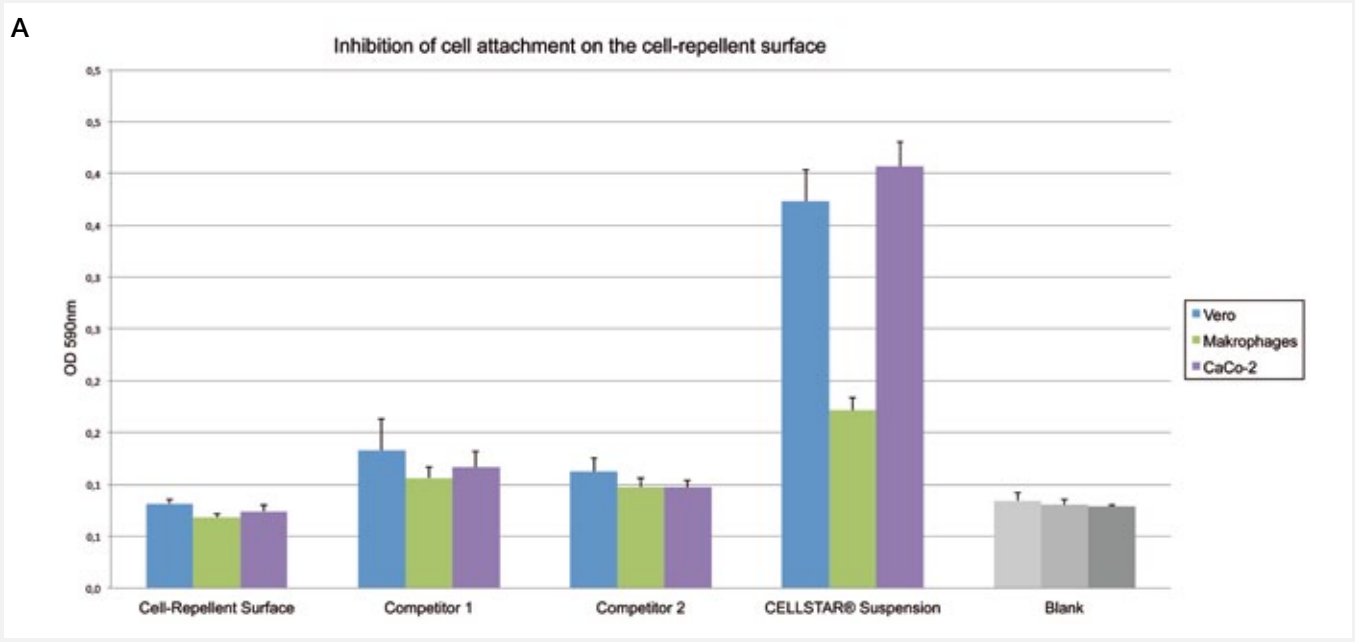


Figure 1: No adherent cells are present on vessels with cell-repellent surface.
A: Cell culture dish with cell-repellent surface after incubation with EtOH.
B: Cell culture dish with CELLSTAR® TC surface after incubation with EtOH.

3. Inhibition of cell attachment of semi-adherent and adherent cell lines in vessels with cell-repellent surface

For the culture of suspension cells, surfaces of a strong hydrophobic nature are generally used. With semi-adherent cell lines like macrophages or even adherent cell lines like Vero or CaCo-2 cells, this kind of surface does not reliably prevent cell attachment.

However, if cultivated in vessels with a cell-repellent surface, these cell lines exhibit an almost total inhibition of attachment (Fig. 2). Tested cell lines are listed in Table 1.



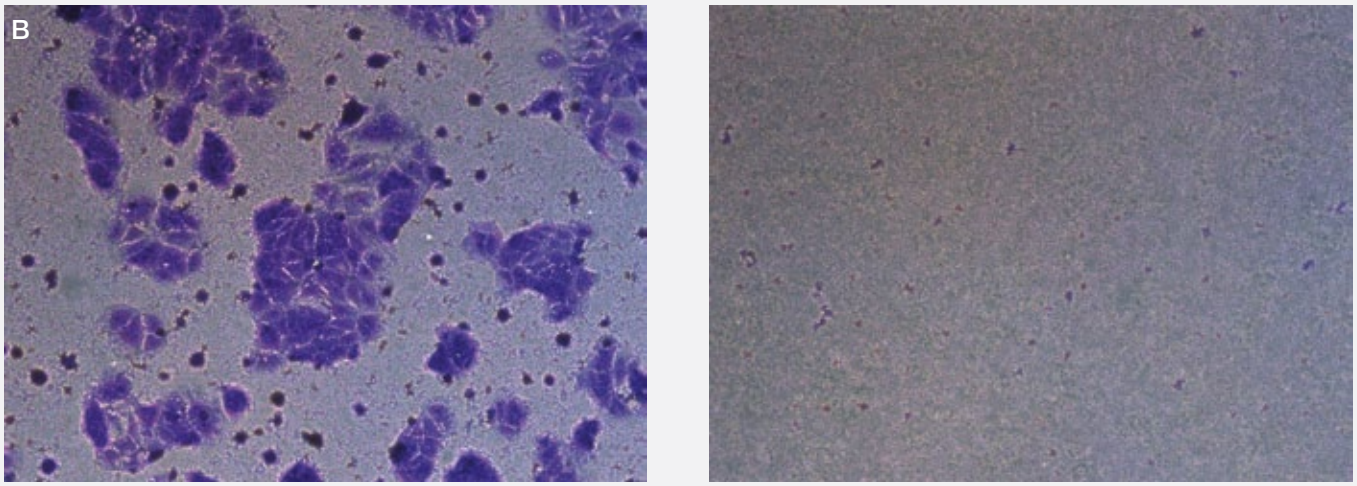


Figure 2: The cell-repellent surface inhibits cell attachment effectively. **A)** Spectroscopic analysis of cell attachment of Vero, CaCo-2 cells and macrophages. Cells were seeded in F-bottom 96 well microplates, incubated at 37 °C and 5 % CO_2. After 24 hours the media were discarded. Cell attachment was analysed by crystal violet staining. After dissolving the crystal violet dye in the cells attached to the well surface, optical density was measured at 590 nm. **B)** Microscopic analysis with 10 x magnification after crystal violet staining (Left: CELLSTAR® suspension; Right: cell-repellent surface).

4. Culture of spheroids and stem cell aggregates

In pharmaceutical and basic research, two-dimensional (2D) cultures are still predominant. Nevertheless, 2D cultures can only mimic to a limited extent the conditions in physiological tissue where cells are able to interact in a three-dimensional network. Therefore, results generated from 2D cultures have often limited relevance for clinical effectiveness [1].

The employment of spheroid cultures plays an important role as an alternative approach to better mimic physiological conditions, especially in cancer research.

Spheroids are self-assembled spherical cell clusters with different zones (Fig. 3). These zones are characterised by metabolic and proliferative gradients resembling the physical conditions in avascular tumours or micrometastases [2,3]. As a consequence, experimental data obtained with spheroids are often more significant than data from 2D cell culture experiments.

Pluripotent stem cells with their ability to differentiate into cell types of all germ layers (endoderm, mesoderm and ectoderm) [4] hold great promise for drug development, therapeutic applications as well as for basic research.

A key step for the cultivation of stem cells is the formation of non-adherent cell aggregates, called embryoid bodies [5].

Pluripotent cell types which are able to form embryoid bodies comprise both embryonic stem cells and induced pluripotent stem cells.

Application	Tested Cell Lines
Cell Adhesion	Vero MDCKII alveolar Macrophages (rat) CaCo-2 Jurkat CHO SK-N-MC STO-DA
Stem Cell Aggregate Formation	mES-D3 human iPSCs
Spheroid Culture	HeLa HepG2 LNCaP HEK-239

Table 1: Tested cell lines

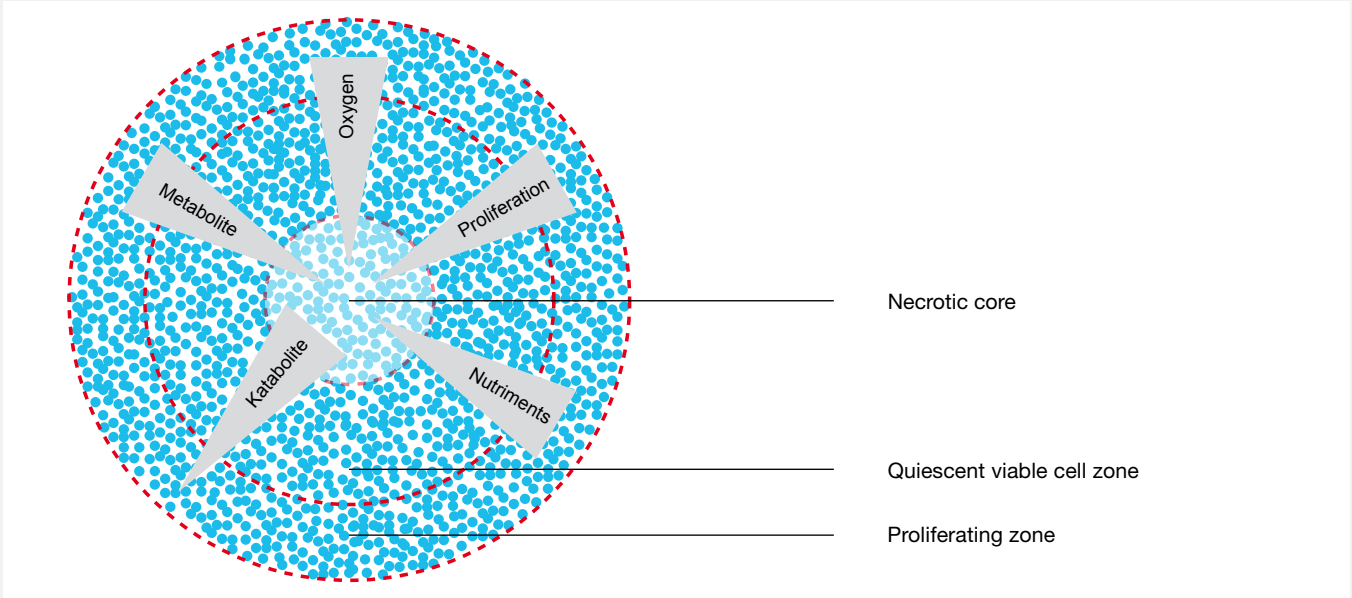


Figure 3: Schematic description of a tumour spheroid

For the formation of spheroids and embryoid bodies cell-cell interaction has to be dominant over the interaction of the cells with the surface of the culture vessel used. Standard cultivation approaches comprise the hanging drop method, spinner flask or rotary cell culture for spheroid cultivation [1] and static suspension culture for embryoid bodies. However, these approaches have only limited compatibility with automation and high throughput screening.

On the other hand, 'classic' cell culture vessels like microplates fulfil all the necessary demands for automated handling and imaging. If equipped with a surface effectively preventing cell-surface interactions like the Greiner Bio-One cell-repellent surface these vessels represent a perfect platform for cultivating spheroids (Fig. 4) and stem cell aggregates (Fig. 5, 6).
Tested cell lines for spheroid and aggregate formation are listed in Table 1.

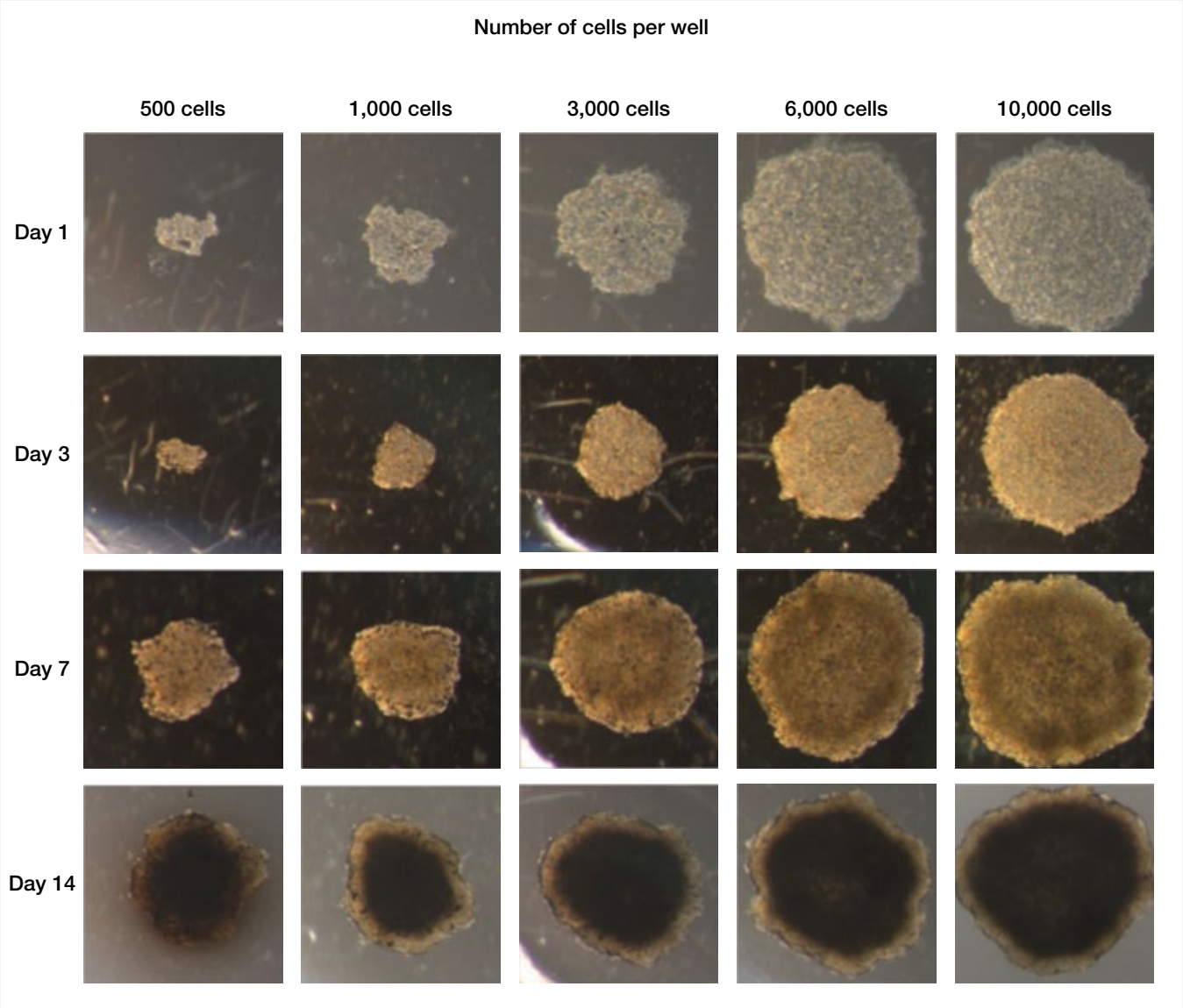


Figure 4: LNCaP cells form single spheroids in 96 well U-bottom plates with cell-repellent surface. Their size is dependent on the number of cells used for seeding.

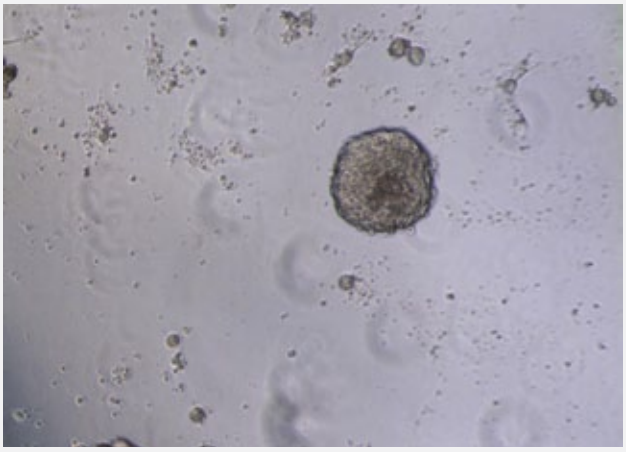


Figure 5: Aggregates of murine ES-D3 cells grown for 10 days in a 96 well F-bottom microplate with cell-repellent surface. Before the transfer to the cell-repellent surface, cells were cultivated on feeder cells and detached with accutase.

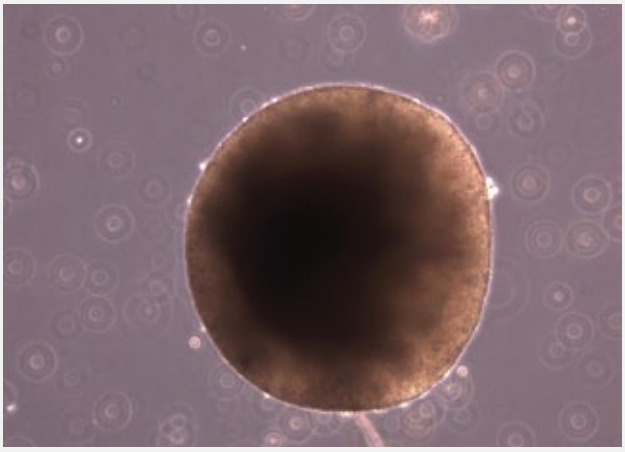


Figure 6: Aggregate formation of human induced pluripotent stem cells (iPSCs) cultured in a 96 well U-bottom microplate with cell-repellent surface. Before the transfer to the cell-repellent surface, cells were cultivated on Matrigel™ and detached with accutase.

5. Ordering Information

Cat. No.	Description	Quantity per bag	Quantity per case
655 970	96 well microplate, PS, F-bottom/chimney well, clear, cell-repellent surface, sterile, with lid	1	6
650 970	96 well microplate, PS U-bottom, clear, cell-repellent surface, sterile, with lid	1	6
657 970	6 well multiwell plate, PS, clear, cell-repellent surface, sterile, with lid	1	5
628 979	Cell culture dish, ⌀ 60 x 15 mm, PS, clear, cell-repellent surface, sterile	10	20
664 970	Cell culture dish, ⌀ 100 x 20 mm, PS, clear, cell-repellent surface, sterile	1	5

Additional formats are available upon request.

6. Literature

[1] Friedrich J. et al. (2007). Experimental anti-tumour therapy in 3-D: Spheroids - old hat or new challenge? Int J Rad Biol. 83(11-12):849-871.

[2] Friedrich J. et al. (2009). Spheroid-based drug screen: considerations and practical approach. Nat Protoc. 4(3):309-24C.

[3] Kunz-Schughart L. A. et al. (2004). The use of 3-D cultures for high-throughput screening: the multicellular spheroid model. J. Biomol Screen. 9(4):273–285.

[4] Itskovitz-Eldor J. et al. (2000). Differentiation of human embryonic stem cells into embryoid bodies comprising the three embryonic germ layers. Mol Med. 6:88-95.

[5] Höpfl G. et al. (2004). Differentiating embryonic stem cells into embryoid bodies. Methods Mol boil. 254:79-98.

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