PrimeCoat
- PRODUCT DESCRIPTION -

01/01/16

The ExCellness PrimeCoat series is designed specifically to provide a biomimetic cell culture environment that improves cell characteristics and phenotype in laboratory applications.

Main applications:

- PrimeCoat substrates are used to model healthy and diseased tissues *in vitro*.
- PrimeCoat substrates are used to determine the optimal mechanical conditions for cells to express protein of interest appropriately.
- PrimeCoat substrates are used to determine the effect of substrate stiffness on cell proliferation rates, apoptosis, cytokine production, metabolism, etc.

Key Features:

- PrimeCoat substrates are easy to use for cell culture and subsequent analysis.
- PrimeCoat substrates are available with five degrees of softness within the elastic modulus (E) range of body tissues: E=2, 5, 10, 15, 30 and 100 kPa.
- PrimeCoat substrates are available in four standard formats: ∅100 mm dishes, ∅35 mm dishes, 24-well plates and 20x20 mm coverslips.
- PrimeCoat substrates are completely transparent and cells can be visualized with standard transmission light microscopes (e.g., phase contrast, DIC). Minor reduction in optical quality may occur with very soft substrates due to micelle inclusions in the bulk material. These inclusions do not affect the mechanical substrate properties.
- PrimeCoat substrates are compatible with most standard molecular or cellular techniques: immunofluorescence, immunohistochemistry, protein analysis (e.g., Western blotting, and RNA/DNA extraction).

Special comments regarding "PrimeCoat" items:

"PrimeCoat" products represent the latest technology of ExCellness Biotech SA. The clean (but non-sterile) products have been tested for cell culture.

Shipping:

20x20 mm 0.18 mm thin PrimeCoat cover slips are shipped on an adhesive surface. To detach PrimeCoat substrates from the surface use Dumont No.5 forceps. Be patient to not break the glass support.

Storage:

PrimeCoat substrates are stored at RT. Storage of up to 6 months will have no influence on substrate stiffness. Please use PrimeCoat before the date indicated on the packaging (DLU).

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PrimeCoat
- INSTRUCTIONS OF USE -

Important Note:

PrimeCoat substrates must be coated (please ref. below) by the user to promote cell adhesion.

Sterilization procedure:

- PrimeCoat substrates are provided clean but non-sterile. They can be sterilized by brief washings (3 x 5 sec) with 100% isopropanol.
- Do not expose PrimeCoat substrates to UV light or excessive heat (vapor sterilizer) : both treatments will change the elastic modulus of the material.

Coating Procedure:

- To promote cell attachment, coat substrates with any extracellular matrix protein that promotes attachment of your cells for minimum 1h at 37°C (we recommend overnight coating at 37°C). Optimal conditions for attachment must be determined for each cell line and application. Coating of soft substrates does not differ from that of any other culture ware and detailed protocols can be obtained from the protein vendors.
- Non-coated PrimeCoat substrates are hydrophobic and thus require relatively large volumes of coating protein solution.
- It is recommended to use proteins at saturation, commonly at least 2 $\mu g/cm^2$.
- Example for coating with most proteins (e.g., fibronectin, laminin, vitronectin; **NB: best coating results have been obtained using plasma fibronectin**):
  - Dilute protein stock solution in medium without serum to obtain 2 μg/200 µl solution (10 μg/ml).
  - Coat surfaces with 200 µl/cm^2 (=2 μg protein/cm^2); e.g., one 35 mm dish (~10 cm^2) with 2 ml solution.
- Example protocol for gelatin coating (we recommend to using gelatin rather than collagen type I which has produced variable results for some researchers in the past):
  - Stock solution: Prepare 10 mg/ml gelatin stock solution by dissolving 1 g gelatin in 100 ml tissue culture grade water and sterilize by autoclaving at 121°C, 15 psi for 30 minutes.
    Cool solution down to about 50°C, prepare aliquots of 1.0 ml, and store at 4°C.
  - Dilution: Warm up the 10 mg/ml gelatin stock solution at 37°C in a water bath until the solution becomes clear. Then prepare a 10 μg/ml working solution in cell-culture water.
  - Coating: Immerse surfaces with 200 µl/cm^2 (=2 μg protein/cm^2); e.g., one 35 mm dish or 6-well (~10 cm^2) with 2 ml working solution.
- Immerse substrates for minimum 1h at 37°C (we recommend overnight coating at 37°C).
- Aspirate supernatant (do not rinse) and seed cells.

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Notes:

- Immunohistochemistry: PrimeCoat substrates can be used for any standard immunostaining technique using MeOH, EtOH, PFA, or acetone fixation. We recommend to add 0.02% TX-100 to all buffers used for antibody dilutions and washing steps.
- Cell Lysis: Cell scraping with rubber policemen to recollect protein, RNA or DNA is possible but, due to the softness of the substrates has to be performed more carefully than on plastic. For very soft substrates, we recommend as alternatives a) cell trypsinization and lysis of the pellet after spinning or b) addition of the lysis buffer to adherent cells and incubation under vigorous rocking. No interference has been reported between the substrate material and standard protein/DNA/RNA extraction buffers.

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PrimeCoat
- TECHNICAL DATA -

Color code:

The value of the substrate's softness is indicated on the device using the following color code.

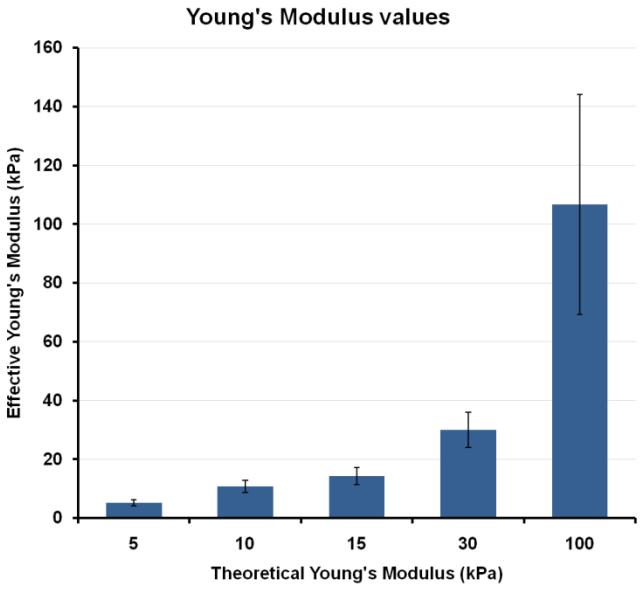
Color code	●	●●	●●●	●	●●	●●●
Softness (E modulus in kPa)	2	5	10	15	30	100

Sterilization:

PrimeCoat substrates are provided clean but not sterilized.

Softness measurement:

The Young's elastic modulus E was determined by rheometry:



Soft substrate thickness :

- ∅100 mm dishes, ∅35 mm dishes and 24-well plate : ~250 µm
- 20x20 mm coverslips: ~30 µm

Notes for microscopy:

- ∅100 mm dishes, ∅35 mm dishes and 24-well plate supports for soft surfaces are standard culture plastic and not optimized for epifluorescence microscopy.
- The thickness of the plastic support (~1 mm) does not allow use of short distance high-resolution objectives. Long distance high-resolution objectives may be used.
- For *in vivo* studies with these formats, use of water-immersion objectives and upright microscopy is recommended.
- For high magnification and inverted microscopy, 20x20 mm coverslips are recommended. Thickness of the glass support and the soft layer together is ~200 µm with a ~500 µm-thick ridge on the edges.

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