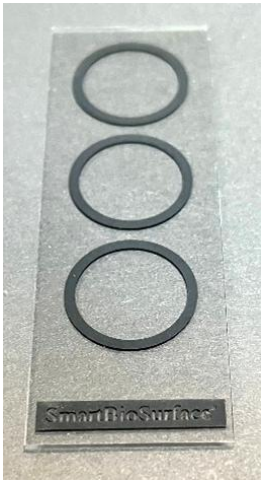
SmartBioSurface® slides (RUO)



Intended Use/Destination of Use (for Research Use Only)

SmartBioSurface® slides ensure spontaneous adhesion (>99%) of otherwise non-adherent cells (tests performed on living white blood cell samples and non-adherent cultured cells in isotonic suspension). They allow the gentle collection of live cells from body fluids or cultured cells in suspension [1].
Centrifugation is not necessary: by simply pipetting a cell suspension, preferably prepared in an isotonic buffer with low or no protein content, onto the slide, after only 10 to 30 minutes of incubation, all cells will be immobilized on the surface with preservation of perfect cell morphology, providing:
- uniform distribution of up to 250.000 live cells per square centimetre
- robust cell adhesion for multiple cycles of staining (bright field [2], ICC, IF [2], FISH [3])
- a sample compatible with all fixation methods
- a sample suitable to be cultured according to standard cell culture methods
- a sample suitable and efficient to be microdissected by laser capture microdissection of single cells

The device does not provide specific results as it is involved in the treatment process aimed at preparing the sample for the next step; therefore, the device is an aid to diagnosis and does not provide any interpretable analytical results.
Slides in an intact box and package have a stability of 18 months.

Instruction For Use

1. Open the slides package and note today's date on the box. The slides must be used within one month from the box opening.
2. Remove the slides from the box and identify the active side, characterized by the black Teflon chamber.
3. The sample deposition area consists of three circular transparent chambers (Ø 15 mm, 177 mm^2 each) confined by a 30 µm thick black Teflon frame and coated with a homogeneous nanostructured titanium dioxide (TiO_2) layer (50 nm), designed to promote cell adhesion (see Fig. 1).
4. Do not touch the surface of the sample deposition areas.
5. Take the slides out of the box only when your samples are ready to be deposited.
6. Label the slides with etiquette if required.
7. According to the next steps (immunostaining or cell culturing) proceed with the following options:
    a. If cells will undergo immunostaining, place the slide on a planar and clean surface
    b. If cells will undergo cell culturing, place the slide in a multiwell (i.e. https://www.sarstedt.com/en/products/laboratory/cell-tissue culture/accessories/product/94.6077.307/); culturing of cells on SmartBioSurface® slides has been tested for up to 72 hours; slides can be trypsinized if needed.
    c. IMPORTANT: Slides are produced in a clean environment but are not sterile; it is suggested to use antibiotics during cell culturing.
8. Pipette the sample in the centre of the active square area:
    a. Minimum volume of the sample is 50 microliters
    b. Maximum volume of the sample is 150 microliters
9. Sample preparation for seeding and adhesion.
   There are two essential conditions for effective adhesion of cells on SmartBioSurface® slides: cells should be alive; the cell suspension should be devoid of proteins or at least have a minimal amount (as in the case of body fluids like Cerebrospinal Fluids, 0.15 to 0.6 milligrams per milliliter).
    a. Blood samples: after Red Blood Cell lysis (using standard commercial protocols based on NH_4Cl buffer, such as https://www.pluriselect.com/it/1x-rbc-lysis-buffer.html#size=61), the WBC pellet should be resuspended in PBS. Please note that for the WBC, the maximum number of cells to be deposited on SmartBioSurface® slides cannot exceed 4×10^5 cells per circular adhesion area (Ø 15 mm, 177 mm^2) [2].
    b. Cultured cells: after cell collection in a falcon tube the cell pellet should be washed twice with PBS and then resuspended in isotonic buffer (i.e. PBS) [4]; after cell wash resuspend the pellet at the concentration of 2.5×10^6 cells/ml and pipette 100–150 microliters onto each circular adhesion area (Ø 15 mm), resulting in approximately 2.5–3.75×10^5 cells per area.
    c. In case of cellular suspension with low cell number, perform, if possible, only one wash step and directly pipette between 50 and 150 microliters onto the adhesion area.
10. Leave the slides undisturbed for at least 20 minutes at RT, allowing the living cells to adhere spontaneously.
11. Check at the light microscope (10x) to evaluate cell density and adhesion and eventually extend the incubation up to 30 minutes at RT; if cells are going to be cultured, after 20 minutes of incubation at RT place the slide for 10 minutes in the 37°C incubator.
12. Remove the supernatant by pipetting.
13. According to the next steps (immunostaining or cell culturing), proceed with the following options:

a. Immunostaining: gently wash with 800 microliters of PBS for 30 seconds, remove PBS, and fix the slides in paraformaldehyde 4%
b. Cell culturing: gently remove PBS from the slide and add the appropriate complete cultured medium in the corner of the multiwell to completely cover the slide, leave undisturbed in the incubator at 37°C overnight.

14. In case of step a) wash the slides in PBS at least 3 times: dry and store at -80 °C or use directly for immunostaining.

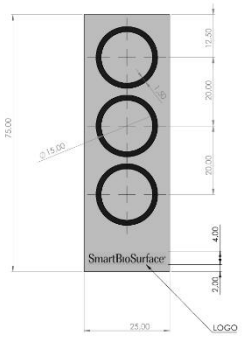


Fig. 1. Graphical representation of the Tethis SmartBioSurface® slide with three circular adhesion areas (Ø 15 mm each).

Fig. 2. Image of a SmartBioSurface® slide with 150 µL of cell solution pipetted onto each of the cell deposition

References

1. Carbone R, Marangi I, Zanardi A, Giorgetti L, Chierici E, Berlanda G, Podestà A, Fiorentini F, Bongiorno G, Piseri P, Pelicci PG, Milani P. Biocompatibility of cluster-assembled nanostructured TiO2 with primary and cancer cells. Biomaterials. 2006 Jun;27(17):3221-9. doi: 10.1016/j.biomaterials.2006.01.056. Epub 2006 Feb 28. PMID: 16504283.
2. Krol I, Schwab FD, Carbone R, Ritter M, Picocci S, De Marni ML, Stepien G, Franchi GM, Zanardi A, Rissoglio MD, Covelli A, Guidi G, Scarinci D, Castro-Giner F, Mazzarella L, Doglioni C, Borghi F, Milani P, Kurzeder C, Weber WP, Aceto N. Detection of clustered circulating tumour cells in early breast cancer. Br J Cancer. 2021 Jul;125(1):23-27. doi: 10.1038/s41416-021-01327-8. Epub 2021 Mar 24. PMID: 33762721; PMCID: PMC8257701.
3. Zanardi A, Bandiera D, Bertolini F, Corsini CA, Gregato G, Milani P, Barborini E, Carbone R. Miniaturized FISH for screening of onco-hematological malignancies. Biotechniques. 2010 Jul;49(1):497-504. doi: 10.2144/000113445. PMID: 20615202.
4. Hayatigolkhatmi K, Soriani C, Soda E, Ceccacci E, El Menna O, Peri S, Negrelli I, Bertolini G, Franchi GM, Carbone R, Minucci S, Rodighiero S. Automated workflow for the cell cycle analysis of (non-)adherent cells using a machine learning approach. Elife. 2024 Nov 22;13:RP94689. doi: 10.7554/eLife.94689. PMID: 39576677; PMCID: PMC11584176.