Instructions for use

Roti®-Bond

CL20.1, CL20.2, CL20.3

Adhesion slides for fast and highly efficient immobilisation of cellular material with optimal structure conservation

I. Introduction

The new adhesive coating of the Roti®-Bond adhesion slides reacts to natural surface structures of cells and tissues, anchoring them securely to the glass surface. Due to this procedure, based on different binding principles, the cells do not loose their antigenicity, their cell functionality and their three-dimensional structure.

In contrary to other coated slides there is no cell loss by using Roti®-Bond adhesion slides even during harsh incubation procedures.

II. Cell-binding

The immobilisation of the cells occurs immediately after contact to the glass surface. Drying of the samples is therefore not necessary. The cellular material can then be fixed with all common fixatives.

At present, there is no evidence of any preferential cell type binding in heterogeneous cellular material.

For optimal adhesive immobilisation of the cells to the surface of the adhesion slides, the cell suspension should be free of culture medium and proteins, because media components could interact with the adhesive coat and reduce cell binding. Cells or tissues should, therefore, be washed and then dropped onto the slides in an isotonic buffer solution (e.g. Roti®-Stock 10 x PBS, Art. No. 1058.1).

III. Cell-cultivation

Cell-cultivation can be continued in appropriate culture medium after cell adhesion, allowing *in-vivo* live assays, e.g. with a multiphoton or laserscan microscope.

As tests have shown, the cultivation on adhesion slides is not compatible to all cell types, because of the strong immobilisation. Particularly cells with great morphologic activity must be tested prior to experiments.

The slides can be sterilised in an autoclave (e.g. 123°C for 30 minutes) or can be alcohol sanitised (e.g. 5 minutes in ethanol 70%) without any loss of binding capacity.

IV. Range of Application

- Immunofluorescence methods or other comparable methods
- Immunoenzymatic tests (Peroxidase, Alkaline Phosphatase)
- Histological staining techniques, e. g. Pappenheim
- Intracellular antigen detection
- Molecular biological tests, e.g. FISH or the detection of specific DNA modifications
- Multiple staining methods
- *In-vivo* live assays with tissues and cells

V. Cell Types

The following kinds of cells can be tested:

- All blood cells such as lymphocytes, monocytes, granulocytes, thrombocytes and erythrocytes
- Cells from bone marrow, effusion, liquor, bronchoalveolar lavage and cell suspension of lymph nodes and tumours
- Cell lines, e.g. hybridoma cell lines, epithelium cell lines from bone and lung, hepatocyte cell lines
- Artificial tissue
- Tissue sections and preparations
- Primary cultures like hepatocytes or tumour cells
- Stem cells, e.g. adult stem cells of bone and brain

VI. Compatibility with Staining Techniques

The adhesion slides are compatible with all current fluorescent and not fluorescent dyes. Particularly tested are

- Fluorescent derivatives, e.g. FITC
- Rhodamine derivatives, e.g. TRITC, Texas Red
- Cy3 and Cy5
- Phycobilli proteins, e.g. PE
- DAPI
- Hoechst 33358 and 3334
- Alexa dyes

VII. Examples of Use

VII.1. Immunohistochemical Assays

In tumour research Roti®-Bond adhesion slides are used for immunohistochemical stains to conserve the structure of cellular material even during harsh incubation procedures, e.g. high temperature denaturation and high pH-values.

In the following example (Fig. 1) the accumulation of platinum DNA adducts (Pt-(GG)) in tissue of inner ear and kidney was analysed, in order to understand the side effects (deafness and affected kidney) of the cytostatic drug *Cisplatin.*

The test was carried out with marginal cells of the inner ear and cells of the proximal tubulus of the kidney. Cryosections of both tissues were applied to Roti®-Bond adhesion slides. Cellular proteins were denatured by alkali treatment (70 mM NaOH, 140 mM NaCl, methanol 40%, pH 12.8, 5 min, 0°C) and digested first with pepsin (10 min, 37°C) and then with proteinase K (10 min, 37°C). After three-step sandwich immunostaining (final staining with ALEXA FLUOR 488) and DAPI-counterstaining the tissues were analysed and documented by fluorescence microscopy.

Result: The morphological analysis shows completely conserved nuclei with well-defined Pt-(GG) adducts (Fig.1). In spite of harsh incubation procedures the nuclei are still fixed to the slides and can be stained without problems (Fig.1 A, B). Overall, the structure of the tissues is still distinct and detectable.

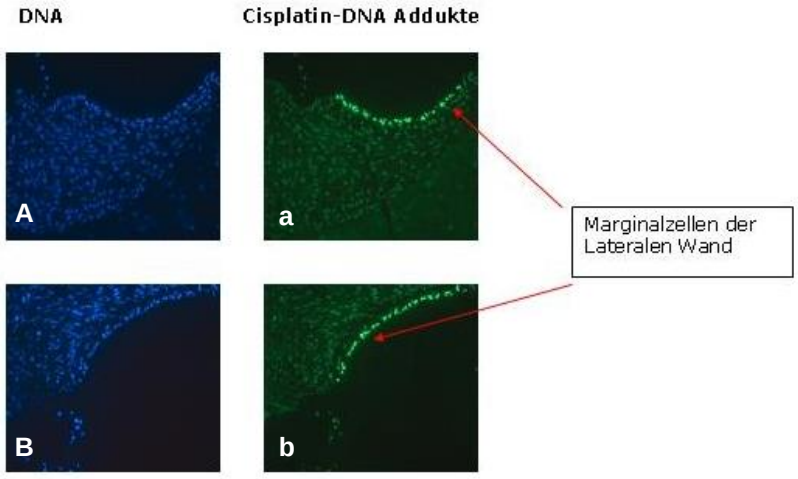

Figure 1:
Presentation of Pt-(GG) adducts in marginal cells of the inner ear of guinea pigs after Cisplatin-treatment. Carrier material of the sections: Roti®-Bond adhesion slides
A, B: Nuclei after DAPI-staining.
a, b: Detection of Pt-(GG) adducts.

With kind assistance of laboratory Dr.J.Thomale, DNA-Repair Group, Institute of Cell Biology, West German Cancer Centre Essen

VII.2. Brand Comparison of Efficient Cell Immobilisation

For assays using precious and only poorly available cellular material (e.g. stem cells) a high recovery rate of the cells following incubation procedures is very important.

Without fixation 1800 murine myeloma cells in 30 µl PBS were applied to SuperFrost™ adhesion slides and Roti®-Bond adhesion slides. Several full immunohistochemical assays (except antibody) were performed in parallel including alkali treatment (70 mM NaOH, 150 mM NaCl, ethanol 40%, 5 min, room temperature) and DAPI-counterstaining of the remaining nuclei. Cells were counted and analysed by fluorescence microscopy and the recovery rates were defined. In Figure 2, two DAPI-stained samples are shown.

™: SuperFrost Company Menzel

Adhesion slide	Cell number in the shown preparation	Recovery rate
SuperFrost	285	15.83 %
SuperFrost Plus Gold	195	10.83 %
Roti®-Bond	1792	99.56 %

Table 1: Recovery rate of murine myeloma cells according to the experiment shown in fig. 2

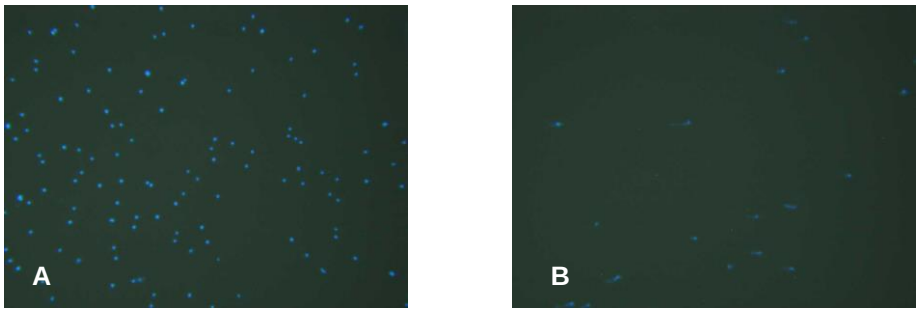

Figure 2:
Brand comparison of cell immobilisation A: Roti®-Bond adhesion slides, B: Superfrost Plus Gold slides. DAPI-staining of the nuclei.

Roti®-Bond adhesion slides	5 Slides	**CL20.1**
	50 Slides	**CL20.2**
	100 Slides	**CL20.3**

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