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FB12 Tube Luminometer
FB12/Sirius Software

Immunoluminescent Method for the Detection of Cell Surface Proteins

This method was established to measure functionally important proteins on the cell surface of tumor cells. In addition, the immunoluminescent technique was applied as an alternative method for the immunofluorescent detection of membrane bound proteins by flow cytometry. Both methods are based on a similar procedure but the immunoluminescence enables a very sensitive and efficient assay technology by indirect protein labeling with peroxidase-coupled secondary antibodies.

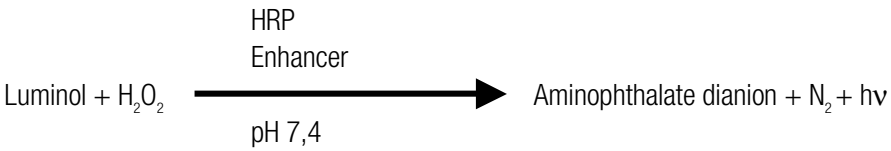
Materials

Luminometer:	Tube Luminometer FB12
Software:	FB12 / Sirius PC Software
Assay:	Immunoluminescence
Tubes:	75 mm x 12 mm ∅

Method

The immunoluminescent technique utilizes primary antibodies and the appropriate secondary antibodies labeled with horseradish peroxidase (HRP). To detect the immunological response, the HRP-catalyzed reaction of luminol with hydrogen peroxide was used. To increase the sensitivity and to optimize the kinetics of light emission, two different enhancers were used: 6-hydroxybenzothiazole or p-iodophenol. Commonly, the procedure comprised the following steps:

- Blocking of IgG and Fc receptors at the cell surface
- Incubation with the primary antibody (60 min, 4°C)
- Washing
- Incubation with the HRP-labeled secondary antibody (45 min, 4°C)
- Washing
- Luminescent reaction



Suitable pretreatments of cells with specific effectors were used to trigger up- or down-regulation of the cell surface protein. Following this modification cell suspensions usually were fixed with paraformaldehyde/PBS (4% PFA, 15 min). Preferably F(ab) or $F(ab)_2$ fragments coupled to HRP were used as secondary antibodies. Aliquots of each labeled cell suspension (50 µl) were mixed with the substrate solution (250 µl) and measured after a delay time of 5 sec. Values reaching a maximum after 20 – 35 sec. were used for the evaluation (single kinetics program).

Example

Detection of the expression level of adhesion molecules and proteolytic enzymes on renal carcinoma cells: renal cell carcinoma cell lines 49RC 43STR and 75RC 2STR, (1).
Specific CD44 isoforms, intercellular adhesion molecule-1 (ICAM-1), urokinase-type plasminogen activator and its receptor (uPA/uPAR) and aminopeptidase N (APN/CD13) are functionally important cell surface proteins involved in invasive and metastatic behaviour of tumor cells. At least two categories of proteins are responsible for invasion and metastasis: adhesion molecules and proteolytic enzymes. Adhesion proteins, as CD44(var) and ICAM-1 mediate cell-cell and cell-matrix interactions and proteases activate proenzymes or initiate whole proteolytic cascades resulting in the hydrolysis of extracellular matrix (ECM) and components of the basement membranes. Among the tumor-associated proteinases and peptidases uPA and APN play a crucial role.

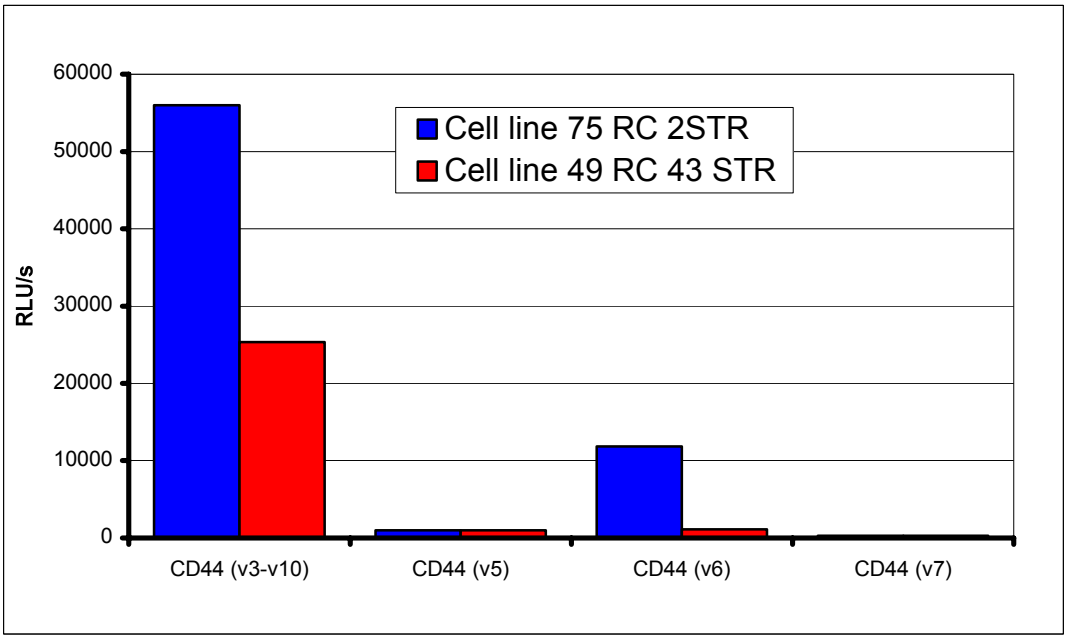


Figure 1. Detection of CD44 variant sequences. Due to more binding sites the polyclonal Ab to CD44(v3-v10) gave the most intensive signal. Only the cell line 75RC 2STR exhibited a strong reaction with anti-CD44(v6) and none of the cell lines displayed a distinct reaction with anti-CD44(v5) or anti-CD44(v7).

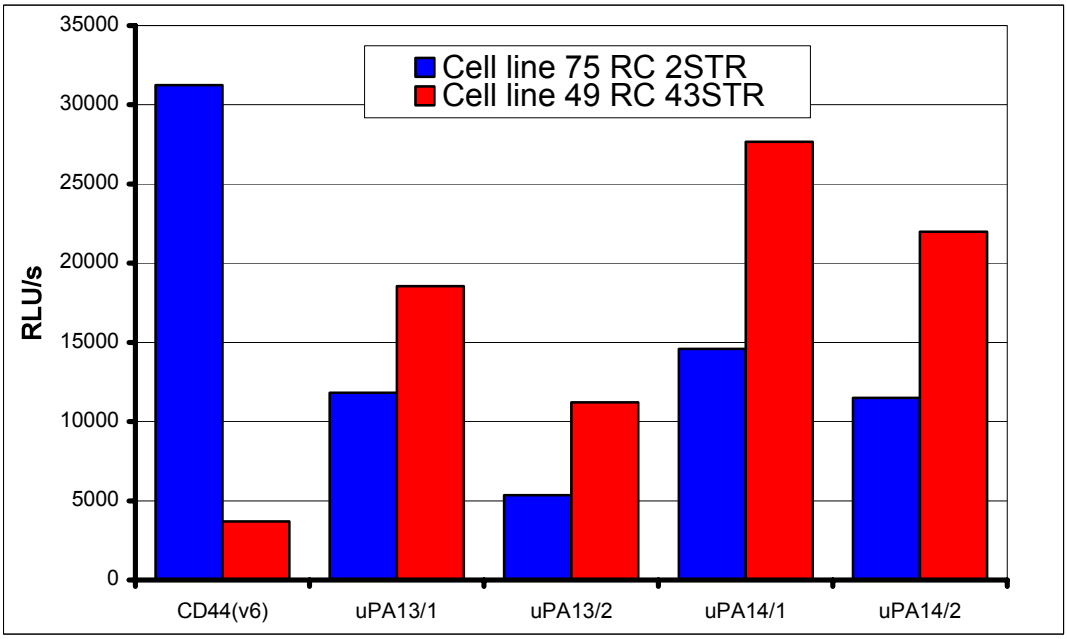


Figure 2. Detection of uPA in comparison with CD44(v6). The signal intensity for CD44(v6) of about 3000 RLU/s (cell line 49RC 43STR) is very low and not significant. 13: anti-uPA (IM13L) recognizes an epitope on the B-chain of uPA. 14: anti-uPA (IM14L) recognizes an epitope on the A-chain of uPA. 1: Ab concentration 10 µg/ml; 2: Ab concentration 2 µg/ml.

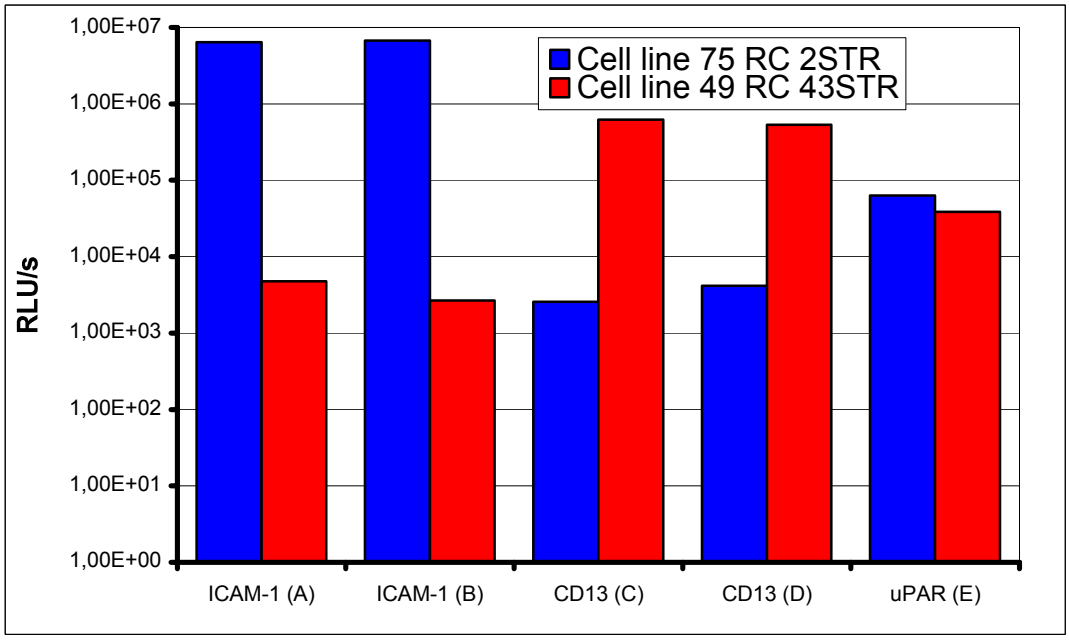


Figure 3. Detection of ICAM-1/CD54, APN/CD13 and uPAR/CD87. (A): anti-CD54 (15.2), (B): anti-CD54 (HA58), (C): anti-CD13 (SJ1D1), (D): anti-CD13 (WM15), (E): anti-uPAR (GR35).

To check the non-specific binding controls were carried out with both normal IgG from the same type and origin as the first Ab and with the corresponding conjugate. Values for the non-specific binding with the former were in the range 1000-2000 RLU/s and with the latter <1000 RLU/s.

Result

The FB12 Tube Luminometer is highly suitable to measure functionally important proteins on the cell surface of tumor cells using the very sensitive and efficient immunoluminescent assay technology.

Acknoledgement
Data provided by Dr. Friedemann Laube, University of Halle, Germany
Figures and text by courtesy of Dr. Friedemann Laube, University of Halle, Germany

Literature

(1) Laube F, Göhring B, Sann H, Willhardt I (2001) Cell surface antigens in renal tumour cells: detection by immunoluminescence and enzymatic analysis. Br. J. Cancer 85, 924-929.