Specific Detection of c-Kit Expressed on Human Cell Surface by Immunosensor Based on Surface Plasmon Resonance

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An immunosensor based on surface plasmon resonance was developed for detection of c-Kit expressed on a cell surface. The combination of the antibody solution modified with gelatin before immobilization to the sensor chip and its blocking with gelatin drastically decreased the nonspecific reaction. The condition may be useful for the detection of various cells by using antibody against cell surface marker including the c-Kit.

Keywords SPR, gelatin, c-Kit, immunosensor, mammalian cell, antibody

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Immunosensor construction

The surface plasmon resonance imaging system (OpenPlex, Horiba Scientific, Palaiseau, France) and the sensor chip made of a glass prism (SPRi-Biochip CS-HD, Horiba Scientific), for which the carboxy group had been esterified with N-hydroxysuccinimide to bind antibodies, were used. The anti-c-Kit antibody and the nonspecific antibody were respectively diluted to 0.2 mg/mL with PBS. The antibody solutions were spotted onto the chip surface area (12 × 23 mm) at each 10 nL/spot using a spotter (Spot Master; Musashi Engineering, Tokyo, Japan) and was immediately left to stand at 4°C for 16 h in 80% relative humidity. The chip surface was then washed 3 times with PBS. It was blocked with PBS containing BSA (10 mg/mL) at 25°C for 1 h and was washed 3 times with PBS.

On the other hand, gelatin solutions (A: 1.0 mg/mL, B: 10 mg/mL) were dissolved at 80°C in PBS and cooled down to 25°C so that they were not gelated. The anti-c-Kit antibody and the nonspecific antibody were respectively diluted to 0.2 mg/mL in PBS with the gelatin A. The antibody solutions were spotted and washed as well, as described above. It was blocked with PBS containing BSA (10 mg/mL) or the gelatin B at 25°C for 1 h and the excess proteins were removed by washing 3 times with PBS.

Unreacted carboxy groups were deactivated with ethanolamine solution (1 mol/L, adjusted to pH 8.5) for 30 min, and then washed 3 times with PBS. The antibody immobilized sensor chip was set into the instrument. The running buffer was prepared at the conditions: PBS containing BSA (2.0 mg/mL) and Tween20 (200 μg/mL) for the sensor chip blocked with BSA or PBS containing gelatin A and Tween20 (200 μg/mL) for the sensor chip blocked with gelatin B. The set sensor chip was rinsed with the running buffer at 25 μL/min until the SPR signal was stabilized.

Detection of the c-Kit expressed on cell surface

The cell suspensions were prepared at 1 × 10⁷ cells/mL with each of the running buffers. The cell suspension, each 200 μL, was injected to the immunosensor, and set to flow at 25 μL/min for 480 s. It was continuously changed to the running buffer, and was further set to flow at 25 μL/min for 480 s. After detection of the signal from the bound cells, the sensor chip was regenerated with roughly crushed gelatin gel (30 mg/mL) at 25 μL/min for 480 s, as described previously. The sensor chip was reused for the next detection after rinsing with the running buffer.

Fig. 1 Detection of c-Kit expressed on cell surface by immunosensor with BSA or gelatin. Time course of the reaction of immobilized anti-c-Kit antibody with MEG01s (A) and HEK293T (B). Blue line shows the results of the condition of immobilization of the antibody without gelatin and blocking of the sensor chip with BSA. Green line shows the result of the condition of antibody with gelatin and blocking with BSA. Red line shows the results of the condition of antibody with gelatin and blocking with gelatin. All lines are shown by the difference between signal intensity of anti-c-Kit antibody and nonspecific antibody. Each data point is the mean of 4 replicates in the same examination; error bars indicate ± SD. SPR image of the reaction at 480 s from the MEG01s (C) and HEK293T (D). c-Kit shows the results with the anti-c-Kit antibody, and control shows the results with nonspecific antibody. BSA shows the results of the same condition as the above blue line. Gel-BSA shows the results of the same condition as the above green line. Gel-Gel shows the results of the same condition as the above red line.
Results and Discussion

Blocking of the sensor chip is an important step for specific detection by immunosensor. BSA was therefore used firstly as a blocking reagent in this study.\textsuperscript{5–9,21} MEG01s, for which c-Kit numbers by each cell were found in this study may be useful for the detection of various cells by using antibody against cell surface markers.

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