

The characterization of the interaction between a ligand and a cell-surface receptor includes several different measurements. Retention measurements show the dissociation of ligand bound to the receptors after the cell culture has been washed. This application note shows how detailed retention measurements can be performed using rotating RIA.

Experimental

Cancer cells (SKOV-3) were seeded in a local part of a cell dish, as indicated in Figure 1. The seeded cells were allowed to attach firmly to the dish surface for at least 12 hours. Prior to the rotating RIA measurement, the cells were incubated with radiolabelled ligand (an antibody binding to the HER2 receptor) for approximately 1 hour, followed by a wash with cell culture medium. After the wash, the dish was placed on an inclined, rotating support. A radiation detector was mounted over the elevated part and registered the intensity as a function of rotational position.

The difference between the radiation intensity of the target cell area and the area opposite to the target cells was measured every minute by rotating the dish for 48 hours. The retention measurement ran unattended and the resulting binding trace was automatically generated during the course of the measurement. The preparation of the measurement included seeding of one cell dish, starting incubation, washing the cell dish, and starting the instrument. About 2 days later, the results were ready for analysis. The measurement was repeated three times.

Results

The resulting retention plot is shown in Figure 2. This particular antibody bound strongly to and dissociated slowly from the HER2 receptor. After 10.6 ± 1.3 hours, 50% of the initially bound antibodies had dissociated from the receptors. After 48 hours, 15-20% of the antibodies were still bound to the receptors (or internalized into the cells). The rotating RIA measurements were repeatable and the results agreed with historical data obtained using a manual protocol (data not shown).

Conclusion

Rotating RIA is a simple and accurate method for performing retention measurements with a minimum of preparatory steps. The workload and use of consumables and reagents are reduced drastically when compared to manual protocols. Furthermore, the radiation intensity of bound antibodies is measured every minute, providing detailed information on the progress of the interaction.

Acknowledgements

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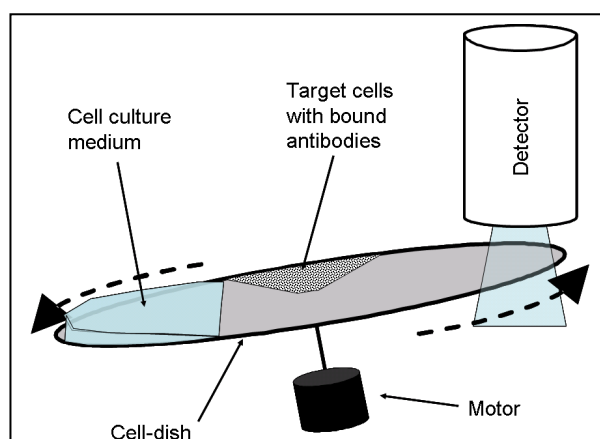


Figure 1. The principle of rotating RIA

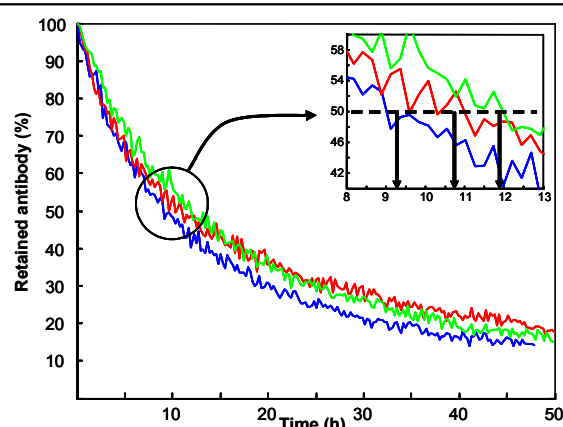


Figure 2. Retention plot of an antibody binding to the HER2 receptor on SKOV-3 cells. The inset shows a magnification of the retention curves near 50% retained antibody.