Sensor Chip CM7
Series S Sensor Chip CM7

BIACORE LABEL-FREE INTERACTION ANALYSIS

Sensor Chip CM7 is designed for screening and characterization of small molecules and fragments in Biacore™ systems. Fragment-based screening is challenging due to the low response levels obtained from these types of analytes. Sensor Chip CM7 has chemical properties similar to Sensor Chip CM5, but with a three times higher immobilization capacity to achieve the required immobilization levels needed to give higher analyte responses.

In addition, the surface may improve immobilization in cases where yields are low due to low preconcentration, acid sensitive ligands, or low activity fractions.

Sensor Chip CM7 provides:

- Higher binding capacity, enabling confident measurements of low molecular weight compounds
- Higher signal-to-noise ratios, improving screening data quality
- Improved chances of success where immobilization levels are unfavorable, such as when target protein has low concentration or is sensitive to optimal immobilization conditions
- The possibility to attach proteins, nucleic acids, carbohydrates, or small molecules
- The possibility for coupling to carboxyl groups on the sensor surface via -NH_2, -SH, -CHO, -OH, or -COOH

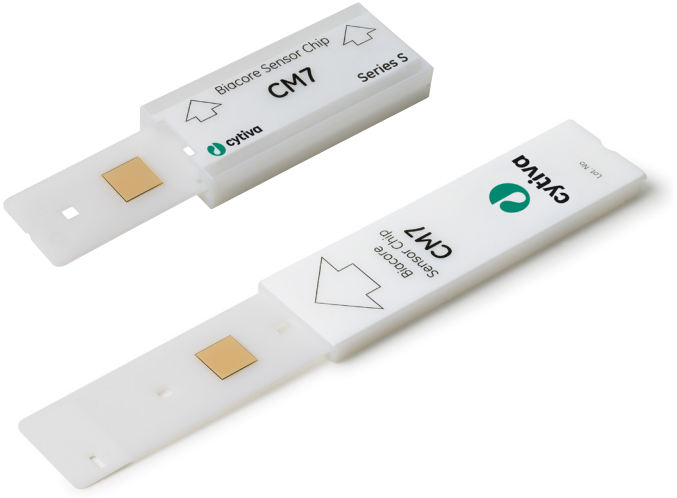

Fig 1. Sensor Chip CM7 and Series S Sensor Chip CM7 have a high immobilization capacity, allowing higher responses for small molecule analytes compared to Sensor Chip CM5.

Description

Sensor Chip CM7 has a carboxymethylated dextran matrix covalently attached to the gold film, with a higher degree of carboxylation and a denser matrix compared to Sensor Chip CM5. The user-defined ligand molecule is coupled covalently to the sensor chip surface via the carboxyl groups, using amine, thiol, aldehyde, hydroxyl, or carboxyl groups on the ligand. The analyte is then passed over the surface and any binding is detected. After binding, the surface can be regenerated either by a regeneration solution or, for reversible binders, by allowing some time to pass between analyte injections.

With macromolecular analytes, it should be noted that the analyte binding capacity (R_{max}) is lower than expected based on the amount of ligand immobilized, possibly because of multi-site attachment of the ligand and a denser matrix compared to Sensor Chip CM5. Sensor Chip CM7 is therefore generally less suitable when working with large molecule analytes.

Improved screening quality

Signal-to-noise ratio, as well as other statistics, is considered to be important for the quality of a screening assay. A wider analytical window between positive and negative results gives higher quality screening assay data and can be described mathematically.

The Z-factor is a universal measure of assay quality. More specifically, it is a measure of the confidence with which positive and negative binders can be discriminated. The Z-factor is calculated from the difference in responses between positive and negative controls in relation to the variation in replicates using the following formula:

$$\text{Z-factor} = 1 - \left(\frac{3 \times (SD_{pos.contr.} + SD_{neg.contr.})}{average_{pos.contr.} - average_{neg.contr.}} \right)$$

A screening experiment with a Z-factor above 0.4 is considered to be acceptable, and a value above 0.8 is excellent.

To test the difference in Z-factor between Sensor Chip CM5 and Sensor Chip CM7, carbonic anhydrase II (CA II) was immobilized using the same immobilization protocol on both chips, resulting in 3700 RU immobilized on Sensor Chip CM5 and 10 700 RU on Sensor Chip CM7. Binding levels from repeated injections of 30 µM furosemide are plotted in Figure 2. Binding levels were increased four-fold, from between 10 and 15 RU on Sensor Chip CM5 to around 60 RU on Sensor Chip CM7. The Z-factor values were 0.42 and 0.84, respectively, demonstrating a significantly improved signal-to-noise ratio. Sensor Chip CM7 thus gave a higher assay quality.

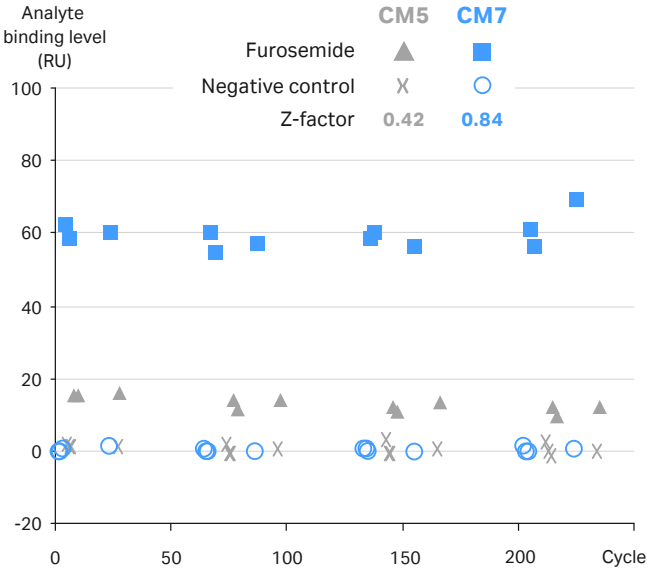

Fig 2. Sensor Chip CM7 gives excellent signal-to-noise ratios as shown here for furosemide interacting with CAII.

Higher immobilization and binding levels

Higher immobilization levels give correspondingly higher responses for low molecular weight compounds, enabling studies in cases where the intrinsically low analyte response is otherwise a limiting factor. Sensor Chip CM7 was compared with Sensor Chip CM5 for three model systems (Fig 3): 30 µM furosemide (M_r = 331) binding to carbonic anhydrase II (CA II), 0.9 mM benzamidine (M_r = 120) binding to α-thrombin, and 30 µM maltose (M_r = 360) binding to maltose binding protein. Using the same immobilization and assay conditions for each chip, 100 µg/ml CA II, 20 µg/ml thrombin and 30 µg/ml maltose binding protein in 10 mM acetate pH 5.0 were immobilized with a standard amine coupling procedure. The results show significantly higher immobilization levels for Sensor Chip CM7, leading to approximately three times higher analyte binding levels compared to Sensor Chip CM5.

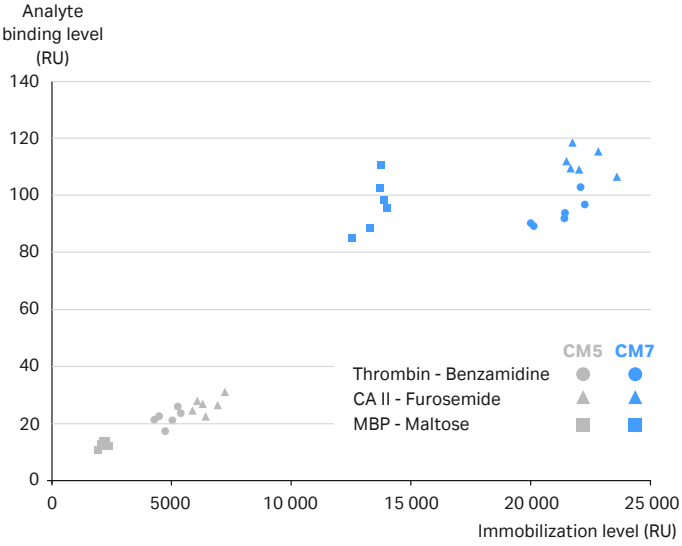

Fig 3. Immobilization and analyte binding levels on Sensor Chip CM5 and Sensor Chip CM7.

Handling unfavorable immobilization conditions

Some protein ligands are difficult to immobilize to sufficient levels on Sensor Chip CM5, for example when the available protein concentration is low, or when a buffer needed to retain protein activity is suboptimal for immobilization. In such occasions, the higher capacity of Sensor Chip CM7 offers the possibility to achieve acceptable binding levels.

CA II was immobilized to Sensor Chip CM5 and Sensor Chip CM7 at low concentration (3 µg/ml in 10 mM acetate pH 5.0; Fig 4A), at high ionic strength (30 µg/ml in 10 mM acetate pH 5.0, 150 mM NaCl; Fig 4B), and at suboptimal pH (30 µg/ml CA II in 10 mM maleic acid pH 6; Fig 4C).

Binding levels from 30 µM samples of sulfanilamide (M_r = 172), furosemide (M_r = 331), and azosulfamide (M_r = 588) were determined in triplicate. The bars in the figure show average binding.

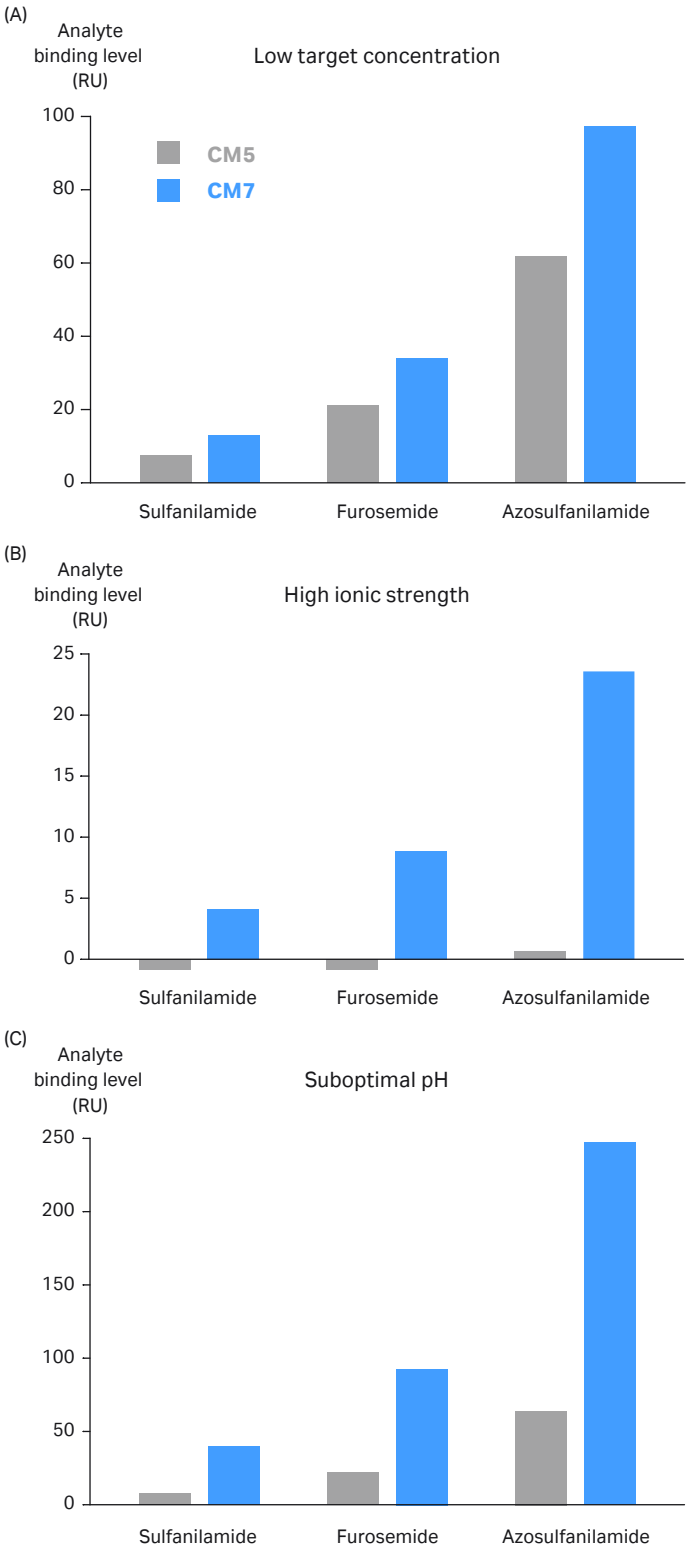

Fig 4. Analyte binding levels on surfaces with CA II immobilized at three different suboptimal conditions on Sensor Chip CM5 and Sensor Chip CM7.

Immobilization in the presence of Tris buffer

Sensor Chip CM7 can also be advantageous when there are interfering substances in the ligand stock solution that compete with the ligand during immobilization. In a situation where the concentration of the ligand protein is low and the amount is limited, a change of buffer may not be an alternative. Tris is a commonly used buffer that is incompatible with immobilization with amine coupling chemistry since the primary amino groups compete with functional groups on the ligand.

The kinase P38 was provided as a 100 µg/ml solution in Tris buffer, and the total volume was not sufficient to allow buffer exchange. After ten times dilution with acetate buffer (10 mM acetate pH 5.5), P38 was immobilized using identical conditions on Sensor Chip CM5 (710 RU) and Sensor Chip CM7 (2240 RU). The final concentration of Tris was 5 mM, and at this concentration, efficient immobilization on Sensor Chip CM5 was impossible while Sensor Chip CM7 immobilized enough kinase for kinetic studies of inhibitor binding (Fig 5).

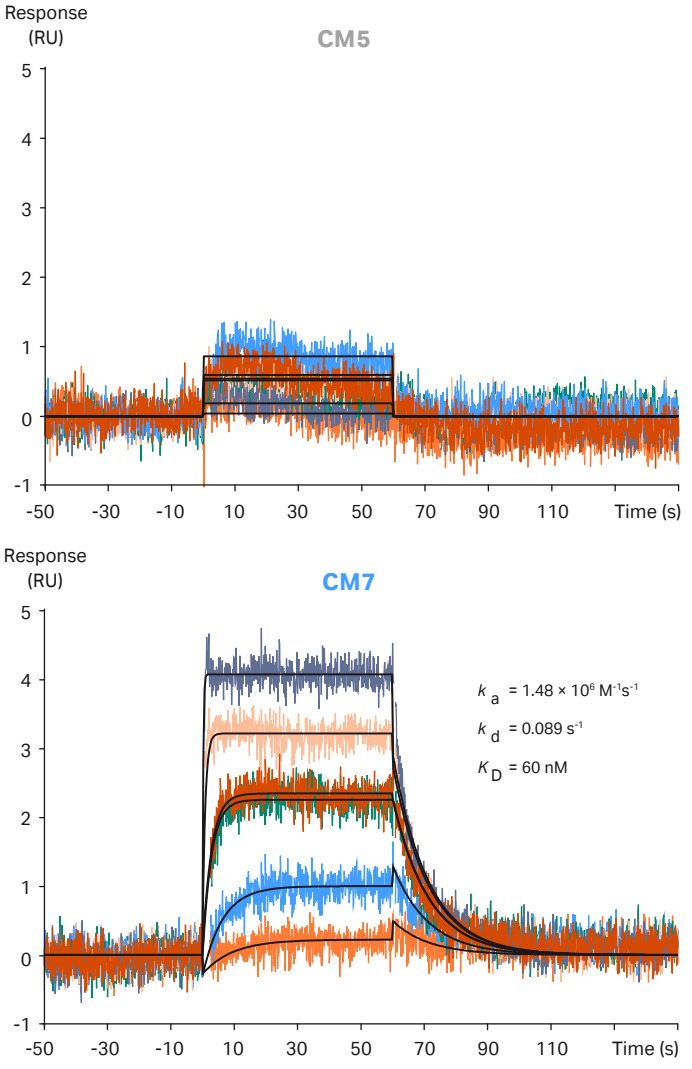

Fig 5. Kinetic experiments with inhibitor SB203580 (concentration series 12 to 3000 nM) binding to active P38α/SAPK2A on Sensor Chip CM5 and Sensor Chip CM7.

Ordering information

Product	Quantity	Code no.
Series S Sensor Chip CM7	Pack of 1	28-9538-28
Sensor Chip CM7	Pack of 1	28-9573-32

Related products

Amine Coupling Kit	BR-1000-50
Amine Coupling Kit, type 2	BR-1006-33
Thiol Coupling Kit	BR-1005-57
Acetate 4.0	BR-1003-49
Acetate 4.5	BR-1003-50
Acetate 5.0	BR-1003-51
Acetate 5.5	BR-1003-52
Borate 8.5	BR-1003-53

Related literature

Sensor Surface handbook	BR-1005-71

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