Application Note #7

iCLUEBIO

Comparison of Storage Kits for Sensor Chip Preservation

Introduction

In SPR analysis, the sensor chip is a core component used to immobilize diverse biomolecules such as proteins, antibodies, and peptides for the quantitative analysis of molecular interactions. These sensor chips are frequently reused for repeated measurements of the same ligand or for long-term projects, rather than being consumed as single-use items. However, the activity of a protein-immobilized sensor chip can deteriorate over time due to denaturation, detachment, or increased non-specific binding; therefore, selecting an appropriate storage method is essential for ensuring reproducibility and reliability.

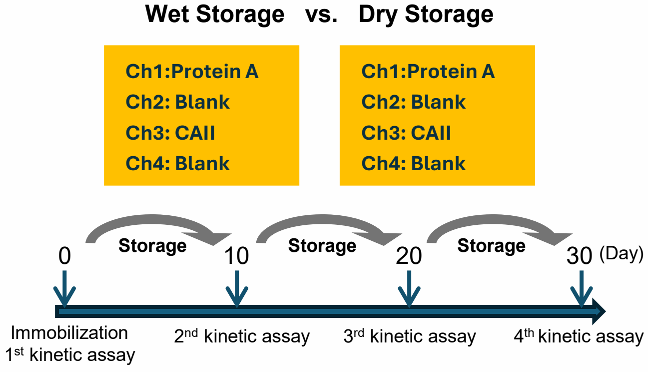
Generally, sensor chip storage methods are categorized into wet storage and dry storage. Wet storage utilizes a dedicated fluidic module to store the ligand-attached surface in contact with a preservation buffer, which is advantageous for ligands that are stable in an aqueous environment. Dry storage maintains the sensor chip surface in a dry state, preventing contamination and microbial growth, and is advantageous for long-term storage and ease of management during storage or transportation.

Icluebio offers two types of storage solutions for sensor chip reuse to accommodate the diverse experimental conditions and protein characteristics of researchers.

Storage Kit – Wet type (CAT# IOSS1000)

Storage Kit – Dry type (CAT# IOSR1000)

This study comparatively evaluated the change in activity and surface stability over time according to the two storage methods (wet vs. dry) using proteins immobilized under identical conditions. This comparison allows researchers to select the most suitable storage method based on the characteristics of ligand and the experimental objectives.



[Figure 1. Schematic Overview of the Experimental Design for Comparing Sensor Chip Storage Methods]

Materials and methods

Materials

- Instrument : iMSPR-Pro2X [CAT#INPX2000]
- Sensor chip : HC1000 [CAT#HCCH101KX]
- Amine coupling kit [CAT#IMAM1000]
- Running buffer: HBST[CAT#RBHTF1010-500]
- Ligand: Protein A, Carbonic anhydrase II(CAII)
- Analyte: IgG , Furosemide
- Regeneration solutions:
  Glycine-HCl (pH 1.5) [CAT#RGGH1015]

Preparation

1. Load the prism holder equipped with the HC1000 chip onto the iMSPR-Pro2X, connect 1X HBST, and proceed with Prime.
2. Perform Precondition by injecting 50 mM NaOH for 3 cycles, 1 minute each, at a flow rate of 20 μL/min.

Protein A – IgG

Immobilization: Protein A

1. Activation: Inject a mixed solution of 200 mM EDC and 100 mM NHS into the ligand and reference channels in continuous mode for 7 minutes at 20 μL/min.
2. Amine coupling: Prepare Protein A at a concentration of 10 μg/ml by dilution in acetate buffer (pH 4.0). Inject it into the ligand channel in Individual mode for 10 minutes at 10 μL/min.
3. Blocking: Switch back to continuous mode and inject 1M Ethanolamine-HCl for 7 minutes at 20 μL/min to block the remaining COOH functional groups.

Kinetic Analysis

1. Prepare the IgG analyte by 2-fold serial dilution in running buffer, as shown in Table 1.
2. Association: Inject the prepared IgG solution for 2 minutes at 50 μL/min.
3. Dissociation: Observe dissociation for an additional 8 minutes while the running buffer flows.
4. Regeneration: After observing dissociation, regenerate under the conditions shown in Table 1.
5. Repeat steps 2 to 3 for the next IgG concentration solution 2-3 times.
6. Immediately after the experiment is complete, check the automatically evaluated kinetic analysis results.
7. The completed sensor chip is stored in the storage kit, and the same experiment is performed every 10 days.

Analyte	Range of analyte conc.
IgG	1.56 - 100 nM
Regeneration solution	**Injection time/ Wash time**
Glycine-HCl (pH 1.5)	1 min / 5 min

[Table 1. IgG Analysis Concentration Range and Regeneration Conditions]

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CAII– Furosemide

Immobilization: CAII

1. Activation: Inject a mixed solution of 200 mM EDC and 100 mM NHS into the ligand and reference channels in Continuous mode for 7 minutes at 20 μL/min.
2. Amine coupling: Prepare CAII at a concentration of 60 μg/ml by dilution in acetate buffer (pH 5.0). Inject it into the ligand channel in Individual mode for 15 minutes at 10 μL/min.
3. Blocking: Switch back to Continuous mode and inject 1M Ethanolamine-HCl for 7 minutes at 20 μL/min to block the remaining COOH functional groups.

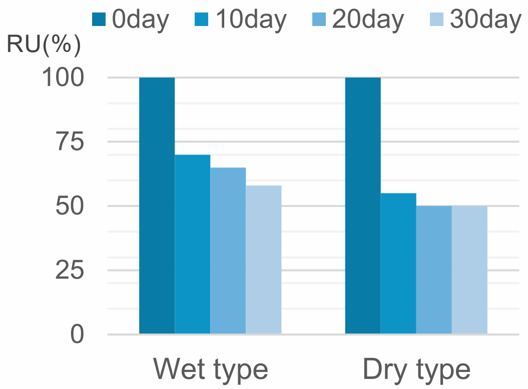
Kinetic analysis

1. Prepare the Furosemide analyte by 2-fold serial dilution in running buffer, as shown in Table 2.
2. Association: Inject the prepared Furosemide solution for 2 minutes at 50 μL/min.
3. Dissociation: Observe dissociation for an additional 6 minutes while the running buffer flows.
4. Repeat step 2 for the next Furosemide concentration solution.
5. Immediately after the experiment is complete, check the automatically evaluated kinetic analysis results.
6. The completed sensor chip is stored in the storage kit, and the same experiment is performed every 10 days.

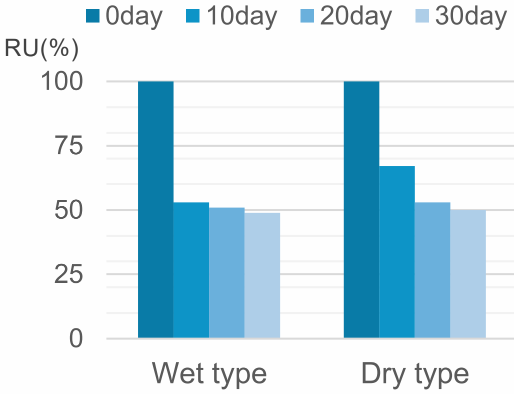
Analyte	Range of analyte conc.
Furosemide	0.156 - 10 μM

[Table 2. Furosemide Analysis Concentration Range]

Results and Discussion



[Figure 5. Protein A–IgG Binding Signal According to Sensor Chip Storage Method (IgG Conc.: 100 nM)]



[Figure 6. CAII–Furosemide Binding Signal According to Sensor Chip Storage Method (Furosemide Conc.: 10 μM)]

Storage kit type	Period (day)	K_{on} ($M^{-1}s^{-1}$)	K_{off} (s^{-1})	K_D (M)
Wet	0	6.24E+04	9.67E-05	1.50E-09
	10	4.25E+04	1.07E-04	2.51E-09
	20	5.50E+04	1.01E-04	1.84E-09
	30	8.18E+04	1.63E-04	1.99E-09
Dry	0	8.24E+04	1.15E-04	1.40E-09
	10	2.76E+04	9.73E-05	3.53E-09
	20	6.48E+04	1.39E-04	2.14E-09
	30	6.54E+04	1.13E-04	1.73E-09

[Table 3. Kinetic Analysis Results of Protein A-IgG]

Storage kit type	Perio (day)	K_{on} ($M^{-1}s^{-1}$)	K_{off} (s^{-1})	K_D (M)
Wet	0	1.35E+04	1.42E-02	1.06E-06
	10	2.02E+04	3.45E-02	1.70E-06
	20	1.72E+04	2.48E-02	1.72E-06
	30	2.69E+04	4.71E-02	1.75E-06
Dry	0	1.59E+04	1.69E-02	1.06E-06
	10	1.38E+04	1.82E-02	1.32E-06
	20	1.26E+04	1.59E-02	1.26E-06
	30	1.22E+04	1.41E-02	1.16E-06

[Table 4. Kinetic Analysis Results of CAII-Furosemide]

Protein A and CAII were covalently immobilized onto identical sensor chips, followed by storage under wet and dry conditions for 30 days. Binding signals and kinetic characteristics were measured at 10-day intervals (Figure 1). The Protein A–IgG pair was utilized as a large-molecule binding model, and the CAII–Furosemide pair as a small-molecule binding model. Analytes were injected at fixed concentrations (100 nM IgG and 10 μM Furosemide) to evaluate the change in the activity of the immobilized proteins (Figure 5 and 6).

Both binding models exhibited a rapid initial decrease in the binding signal during the first 0 to 10 days of storage. Subsequently, the decline rate slowed, and approximately 50% of the initial activity was maintained up to the 30-day point. For Protein A, the initial activity loss in the wet storage condition was relatively less than in the dry storage condition. Furthermore, binding activity remained slightly higher in wet storage at the 30-day mark. Conversely, CAII showed a more gradual activity decrease in dry storage, with both storage conditions exhibiting similar activity levels at 30 days. Since the proteins were immobilized via covalent coupling, the observed signal reduction is interpreted as a loss of binding activity due to decreased structural stability over time, rather than protein detachment.

Despite the reduction in the binding signal during sensor chip storage, the kinetic parameters were largely unchanged, confirming only a limited impact on the affinity measurement. This tendency suggests that the activity deterioration during storage may not necessarily involve concurrent changes in k_{on} or k_{off}. Therefore, it is more reasonable to interpret the signal change as a reduction in the population of active ligands participating in binding, resulting from the functional loss of some ligands due to storage conditions, rather than a full denaturation of the protein structure.

However, the structural characteristics, stability, and sensitivity to the storage environment vary greatly among molecules, making it difficult to completely rule out the possibility that the storage method could affect kinetic properties in certain cases. The distinct patterns of activity loss shown by Protein A and CAII under wet and dry conditions demonstrate that the influence of the storage method can vary depending on the characteristics of the analyzed molecule. Consequently, the suitability of sensor chip storage and reuse should be determined by comprehensively considering the characteristics of the applied sample, the experimental objective, and the measurement period. Especially when measuring the affinity of multiple analytes over a long duration, pre-checking the surface condition using a control analyte after storage can aid in securing experimental reliability. This approach is considered a useful strategy for maintaining data consistency and enhancing experimental efficiency in long-term projects and diverse experimental environments.