Biolipidure®

Water soluble polymers for Diagnostics

Outline

1. Necessity of Biolipidure®

2. Efficacy of Biolipidure®
   - 2-1. Suppression of non-specific binding
   - 2-2. Reduction of lot-to-lot variation for non-specific binding
   - 2-3. Stability improvement of antibodies.
   - 2-4. Enhancement of sensitivity

3. Biolipidure® Product line

4. Summary

Advantage for using Biolipidure

	BSA, Casein	Synthetic polymer
Denaturation	Inevitable	None
Lot to Lot variation	Large	Small
Danger of infection	Possible	None

Synthetic polymers

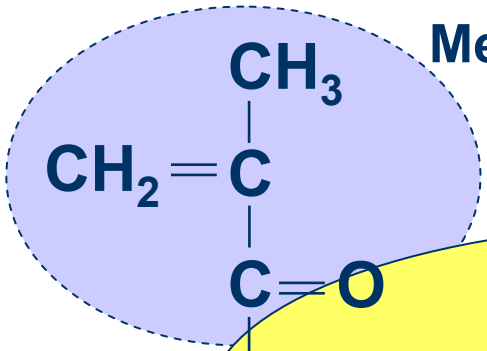
Biolipidure®

Good Stability
Good Reproducibility

MPC is a synthetic monomer

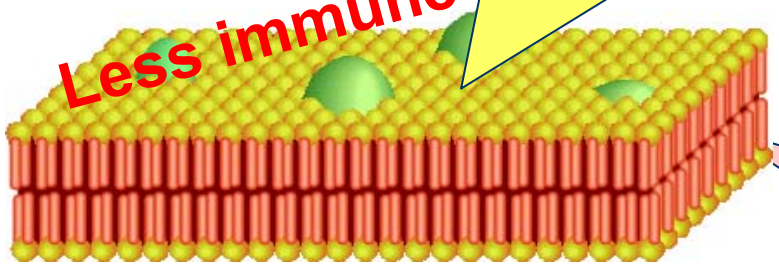
MPC = 2-Methacryloyloxyethyl Phosphoryl Choline

Methacrylic Group



Phosphoryl Choline（PC) Group

Less immune reaction !!



Structure of cell membrane

Phospholipid

PC Feature

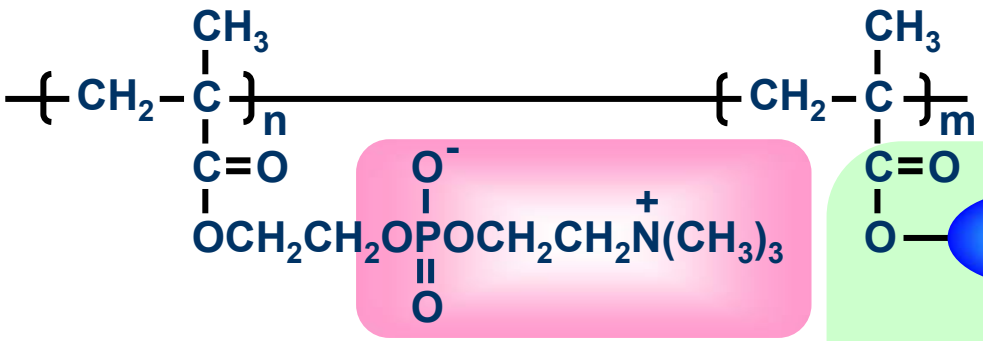
- **Ultrahigh hydrophilicity**
- **Suppression of protein adsorption**
- **Suppression of cell adhesion**

For Antibody

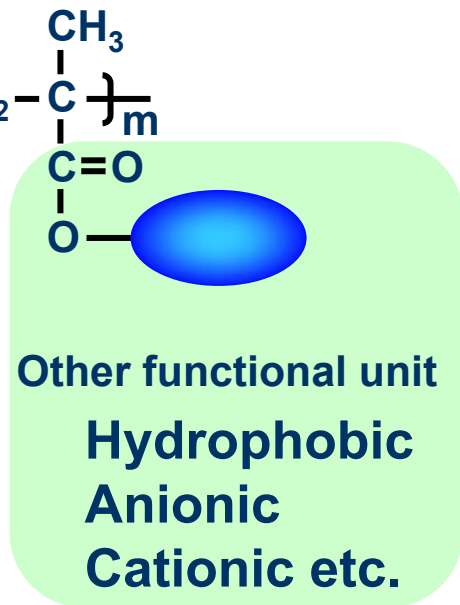
Maintains protein structure

Biolipidure® (Copolymers with MPC)

Structure of Biolipidure®

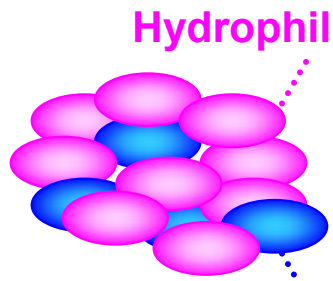


Phosphoryl choline unit



Other functional unit

Hydrophobic
Anionic
Cationic etc.



Structure of Biolipidure in aq. solution

- Appropriate co-monomers can be selected
- Appropriate molecular weight can be chosen
- High purified

Application and Feature of Biolipidure®

For

- **Immunoassay**
- **Western blotting**
- **Immunohistochemistry**
- **Turbidimetric assay**
- **Immunochromatography**
- **Beads based assay**

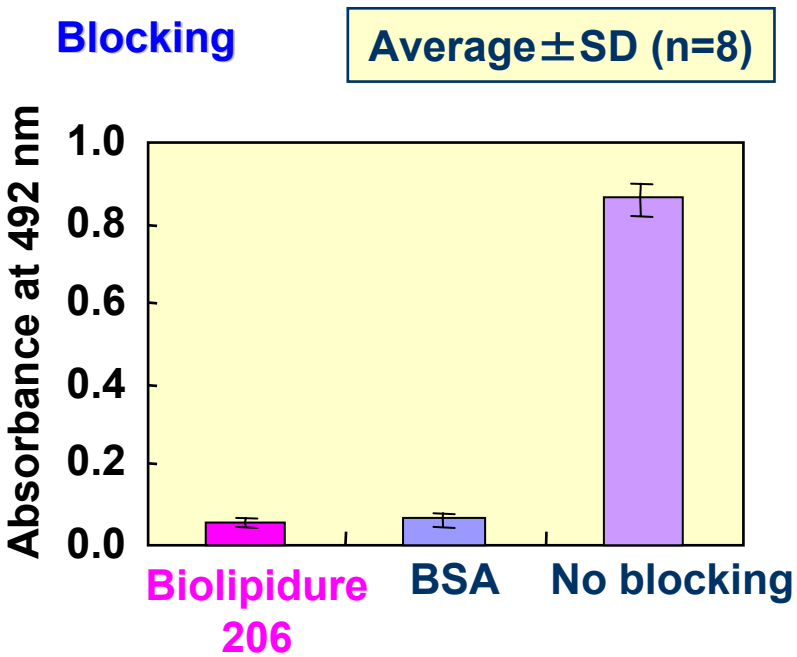
Features

- Suppression of non-specific binding
- Stabilization of immobilized antibody
- Stabilization of enzyme-antibody conjugate in a buffer
- Enhancement of aggregation reaction
- No lot-to-lot variation
- No danger of biohazards

2. Efficacy of Biolipidure®

- *2-1. Suppression of non-specific binding*
- *2-2. Reduction of lot to lot variation*
- *2-3. Stability improvement of antibodies*
- *2-4. Enhancement of sensitivity*
  - *Turbidimetry*
  - *Immunochromatography*
  - *Western Blotting*

2-1. Suppression of Non-Specific Binding



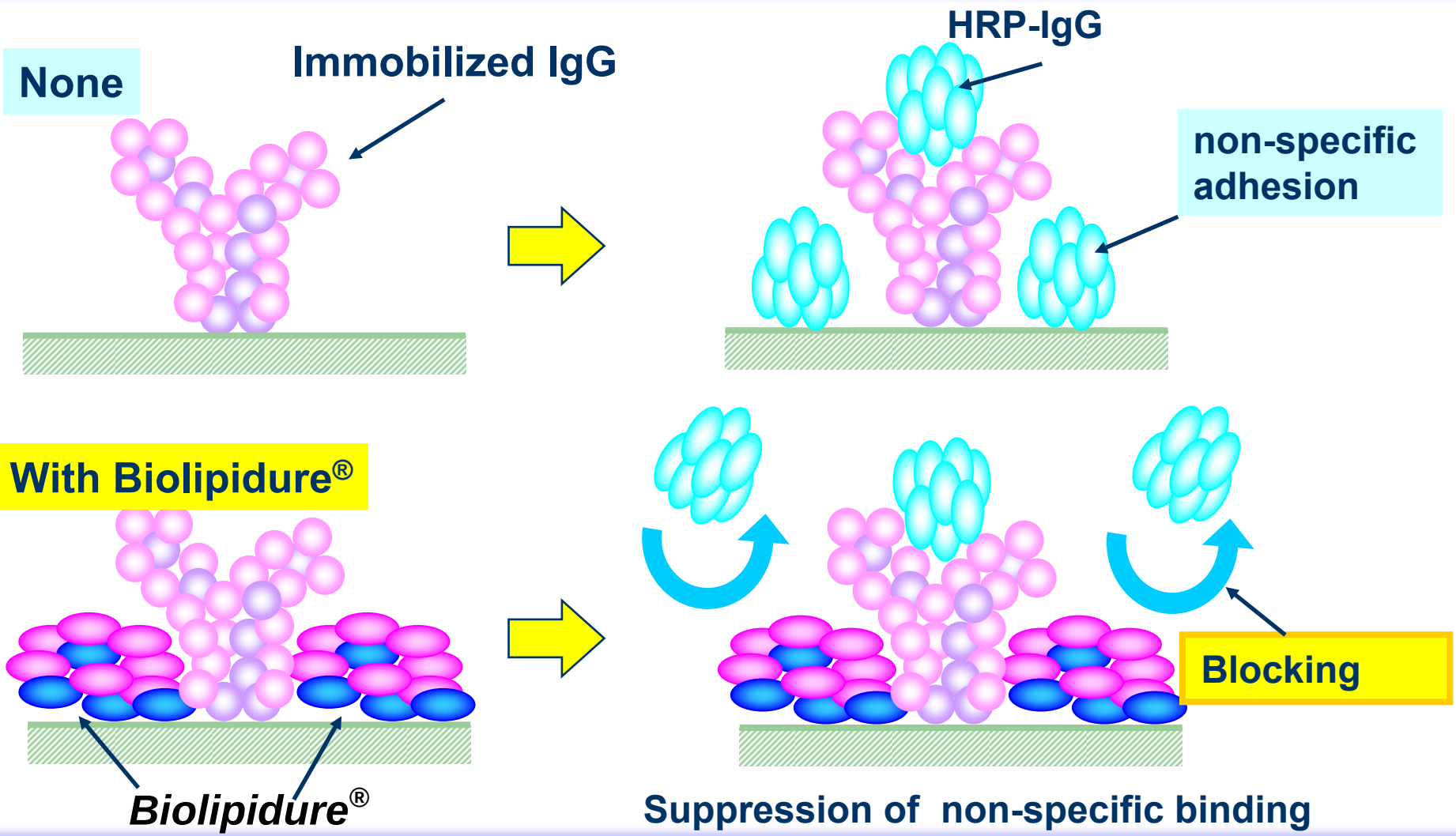
Instrument and sample

- Solid phase: 96-well plate (Maxisorp F96, Nunc)
- Immobilized antibody: anti (mouse IgG)IgG
- Antigen: non
- Enzyme-IgG conjugate: HRP-Anti (mouse IgG) IgG conjugate
- Substrate: *o*-phenylenediamine dihydrochloride
- Detection: 492 nm

Experimental procedure:

(1)Immobilize anti (mouse IgG) IgG
(2)Dispensate 0.1 % polymer (2 % Biolipidure ®-206) and 1 mg/mL BSA for blocking
(3)Dispensate HRP-Anti (mouse IgG) IgG conjugate
(4)Dispensate *o*-phenylenediamine dihydrochloride
(5)Measure absorbance of solution(4) at 492 nm

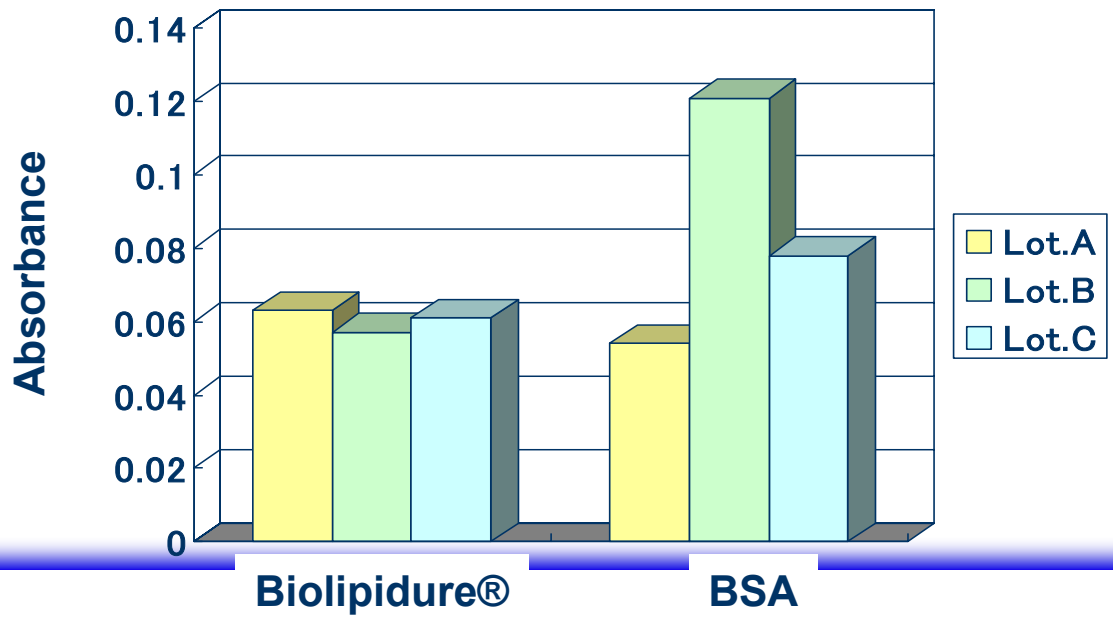
Blocking Mechanism of Biolipidure®



2-2. Suppression of Lot-to-lot Variation

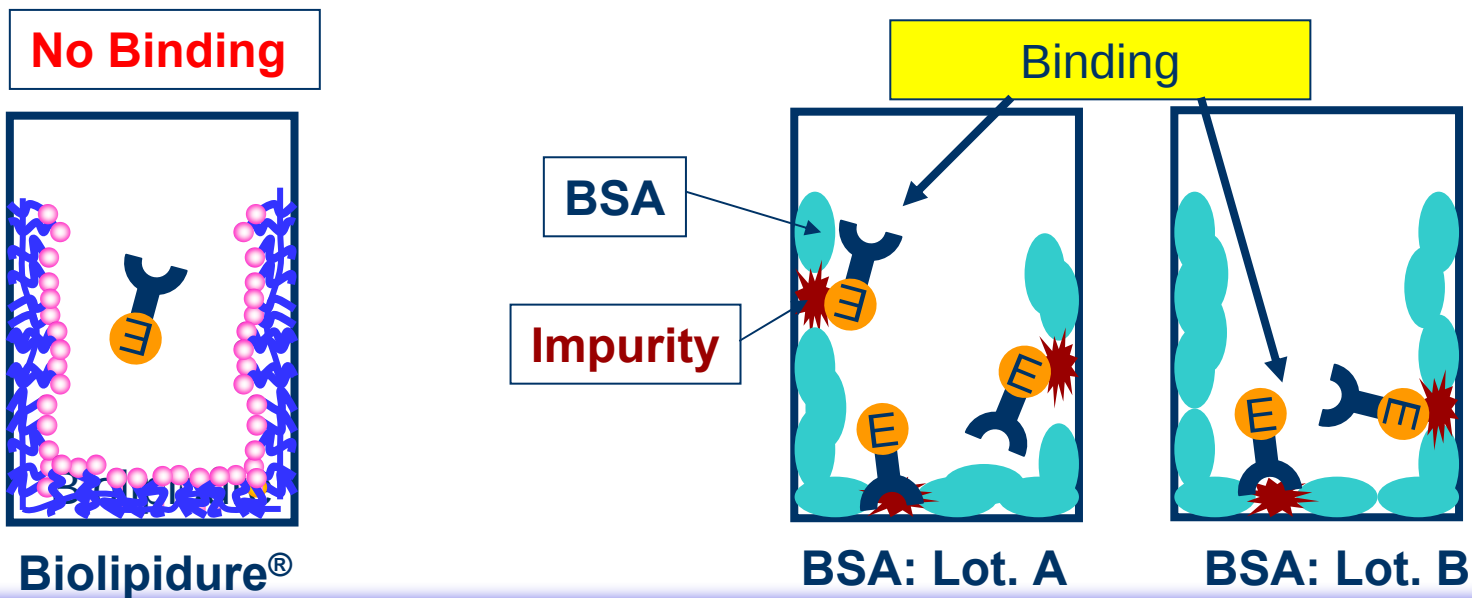
Experiment procedure

(1)Add 200ul of 0.2 % pol ymer (4% Biolipidure®-802) with PB S and 5mg/ ml BSA/ PBS solution in Immunoplate (Nunc Maxisorp F96)
(2)Incubate for 2 hours at room temperature (R.T.)
(3)Rinse each treated plate off with PBS and dry them for 2 hours at R.T.
(4)Add HRP conjugated goat-anti-mouse IgG solution in each well
(5)Incubate for 1 hour at R.T.
(6)Rinse each well off with PBS
(7)Add 3,3',5,5'-tetramethylbenzidine (TMBZ) solution in each well for color reaction
(8)Measure absorbance of the solution at 450 nm



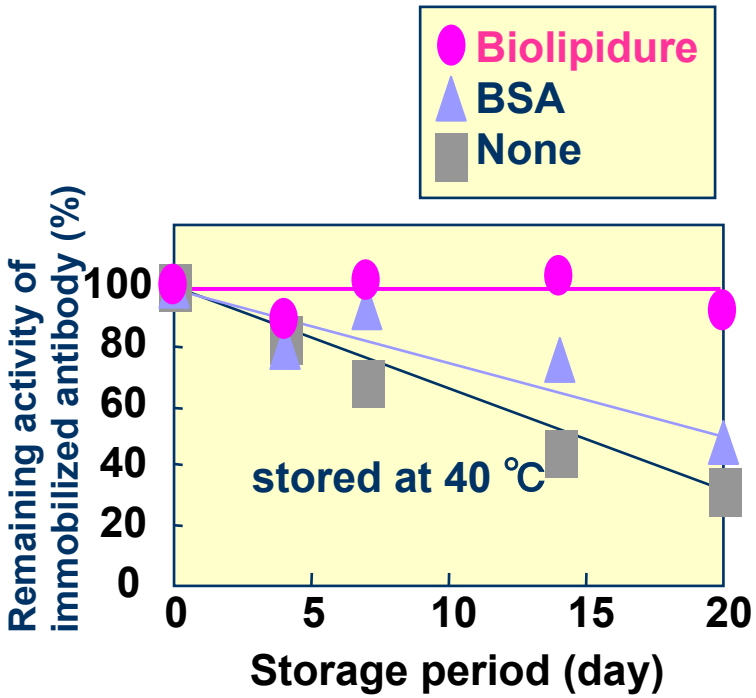
Mechanism: Suppression of Lot-to-lot Variation

Biolipidure®	BSA
➢No impurity ➢Synthetic Polymer	➢Contains impurities ➢Impurity amount is different in each lot
No lot to lot variation	Significant lot to lot variation



2-3. Stability Improvement of Antibody (1)

Stabilization for immobilized antibody



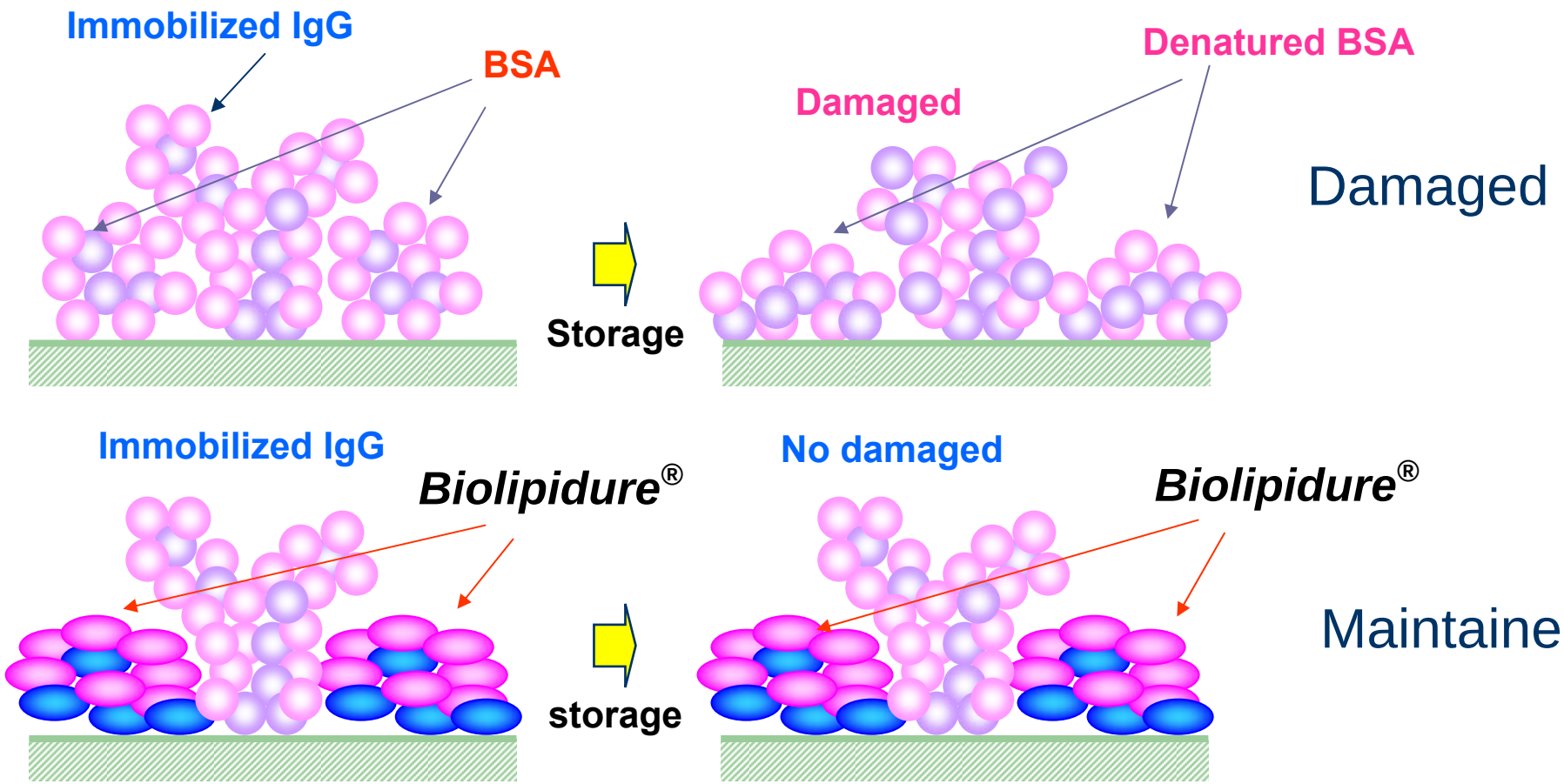
Instrument and sample

- Solid phase: 96-well plate (Maxisorp F96, Nunc)
- Immobilized antibody: anti (mouse IgG)IgG
- Antigen: mouse IgG
- Enzyme-IgG conjugate: HR P-Anti (mouse IgG) IgG conjugate
- Substrate: *o*-phenylenediamine dihydrochloride
- Detection: 492 nm

Experimental procedure:

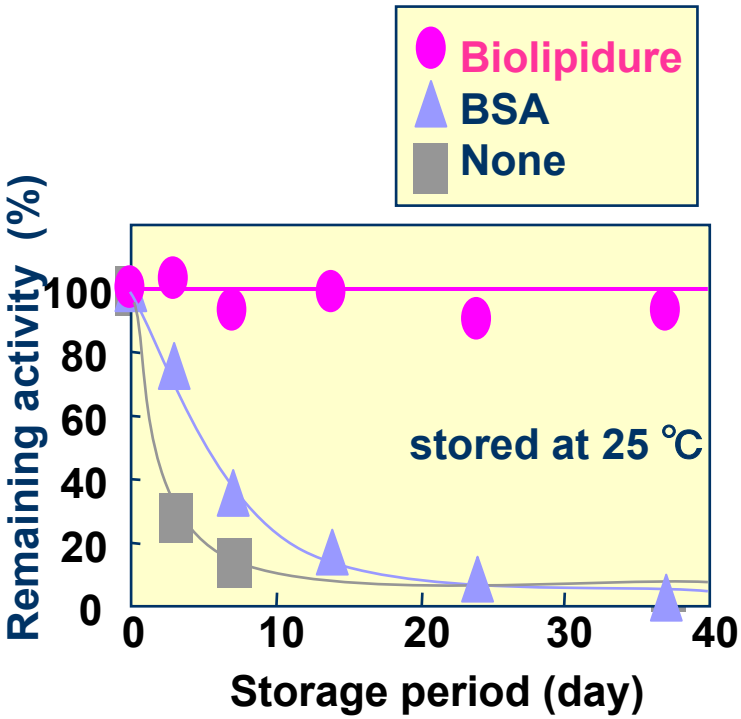
(1)Immobilize anti (mouse IgG)IgG
(2)Add 0.1 % polymer(2% Biolipidure®-802) and 0 .1% BSA for blocking
(3)Suck up the solution in the well and dry
(4)Store at 40 ºC : 0, 4, 7, 14 and 20 day
(5)Dispensate mouse IgG
(6)Dispensate HRP-Anti (mouse IgG) IgG conjugate
(7)Dispensate *o*-phenylenediamine dihydrochloride
(8)Measure absorbance of solution(6) at 492 nm

Stabilization Mechanism of Immobilized Antibody



2-3. Stability Improvement of Antibody (2)

Stabilization for HRP-IgG in a Buffer



Instrument and Sample

Solid phase: 96-well plate (Maxisorp F96, Nunc)
Immobilized antibody: anti (mouse IgG) IgG
Antigen: mouse IgG
Enzyme-IgG conjugate: HRP-Anti (mouse IgG) IgG conjugate
Substrate: *o*-phenylenediamine dihydrochloride
Detection: 492 nm

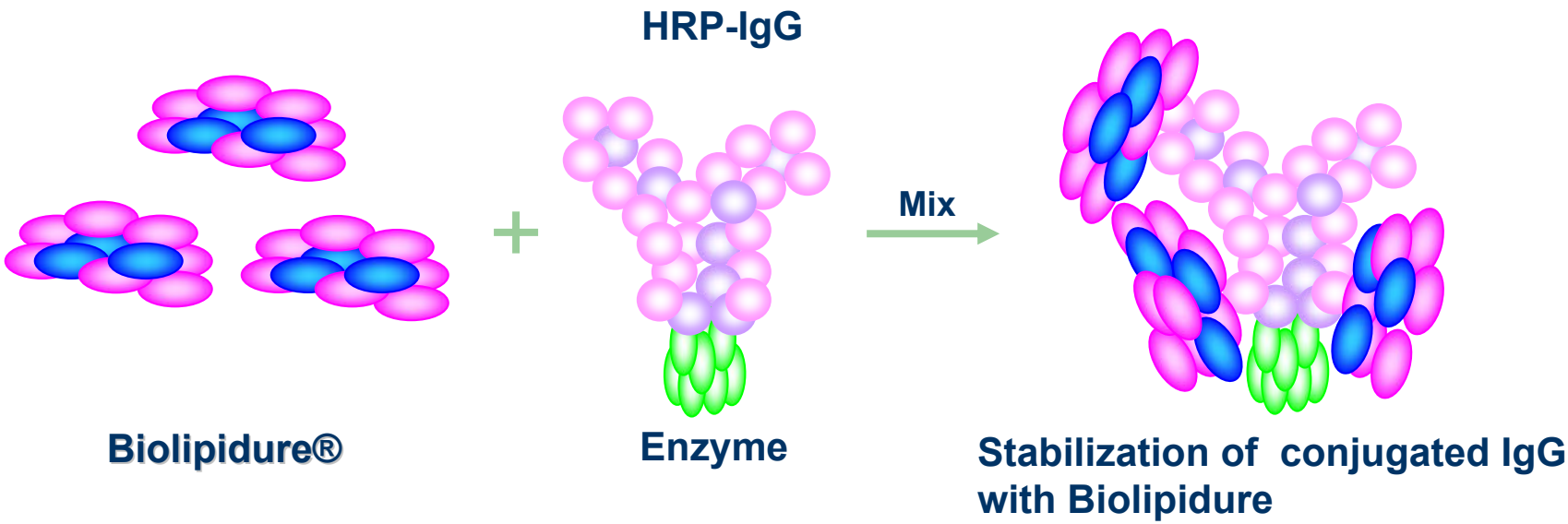
Experimental procedure:

(1)Immobilize antibody and reacted antigen in well plate
(2)Dispensate 1 % polymer (20% Biolipidure®-802 and 1% BSA into HRP-IgG conjugate
(3)Storage at 25 ºC: 0, 3, 7, 14, 24 and 37 day
(4)Dispensate solution prepared at (3) in well prepared at (1)
(5)Dispensate *o*-phenylenediamine dihydro-chloride
(6)Measur absorbance at 492 nm

Biolipidure-802 can stabilize HRP-IgG in a Buffer.

Stabilization Mechanism of HRP-IgG in a Solution

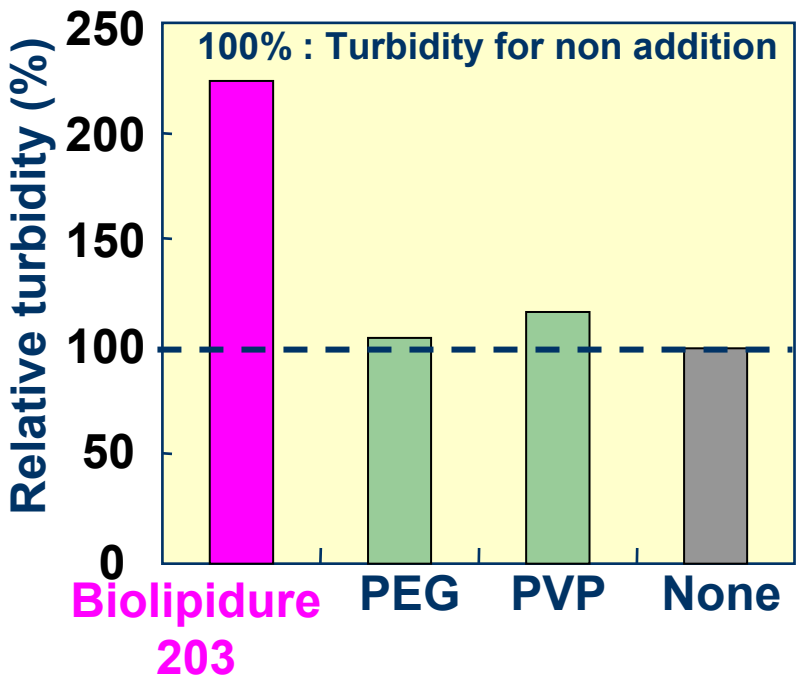
Hydrophilic domains in Biolipidure
Hydrophobic domains in Biolipidure
Hydrophilic domains in Antibody
Hydrophobic domains in Antibody



- Biolipidure® forms a complex moderately with conjugated IgG.
- The conformation of HRP-IgG can be maintained in a buffer.

2-4-1Application for Turbidimetry

Enhancement of aggregation reaction in turbidimetry



PEG : Polyethylene glycol (Mw=6,000)
PVP : Polyvinylpyrrolidone (Mw=40,000)

Instrument and Sample

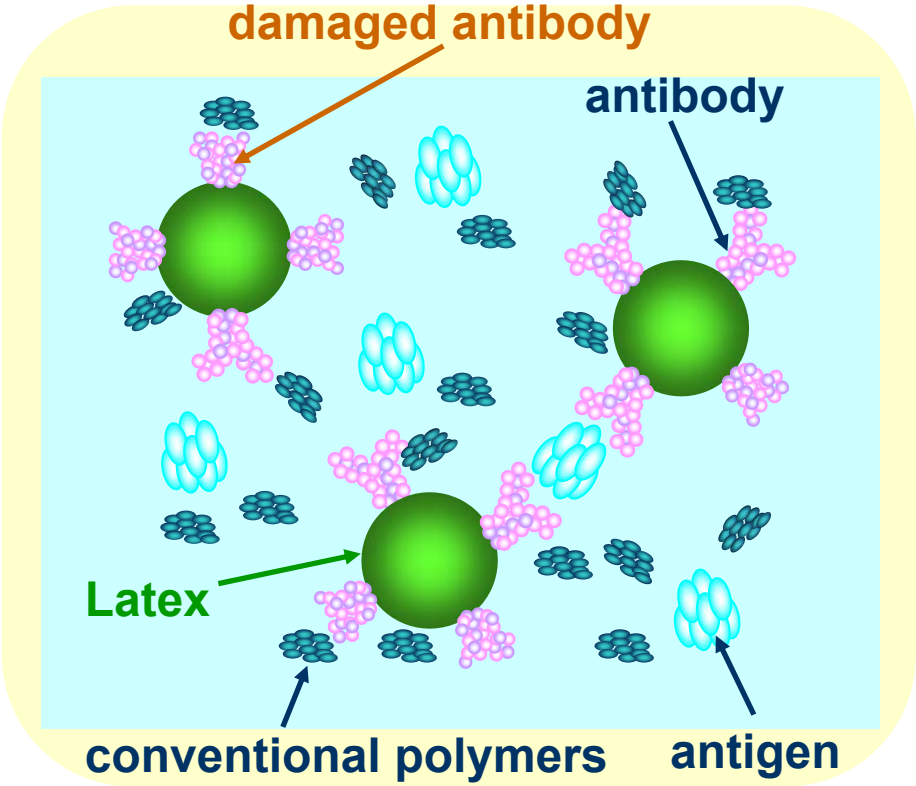
Sample : Human sera
R1 : 1% water soluble polymer buffer solution
R2 : 0.8 mg/dL Anti-CRP ab.-Latex buffer solution
Instrument : 7070Auto Analyzer (Hitachi)
Wave length and : 570nm and 800nm
measurement frequency : 34 times in 10 min

Procedure

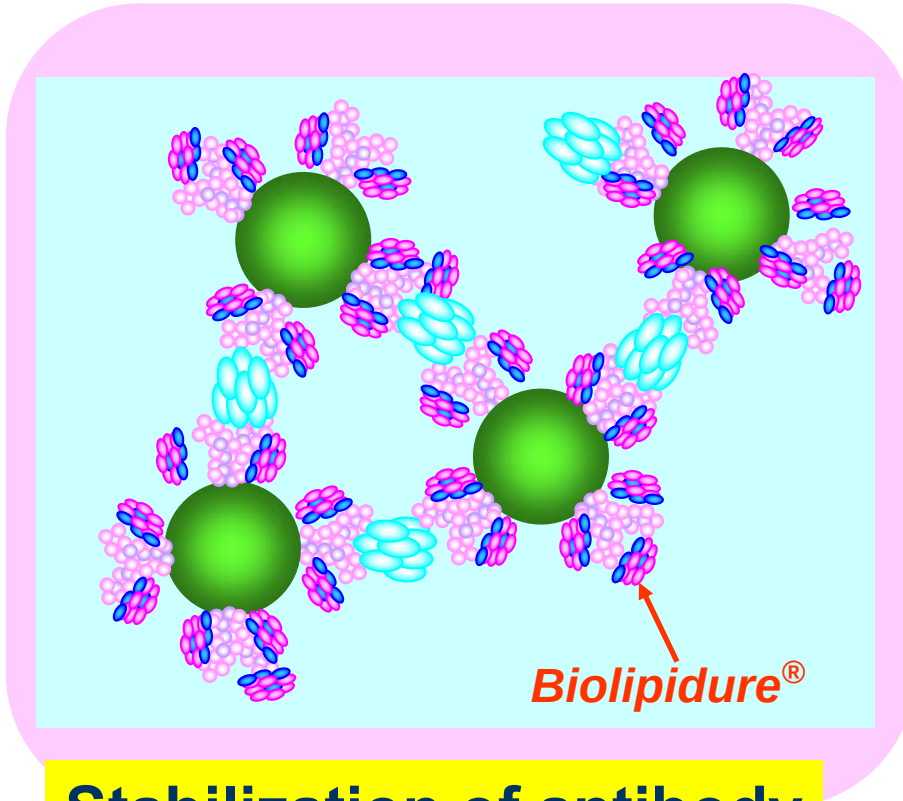
-Sample and all reagents are placed in auto analyzer and distributed automatically, and the absorbance was measured automatically
-The reaction is controlled at 37℃
(1)Dispense 2uL of sample in the cell
(2)Dispense 300ul of R1 on sample(1)
(3)Measure absorbance(ABS1) of solution(2)
(4)5min later, dispense 50uL of R2 on solution(2) and agitate it
(5)Measure absorbance(ABS2) of solution(4)
*In case ABS2-ABS1 is large, the sensitivity of the system is high

Enhancement Mechanism by Biolipidure®

Conventional Polymers



Biolipidure®



Stabilization of antibody

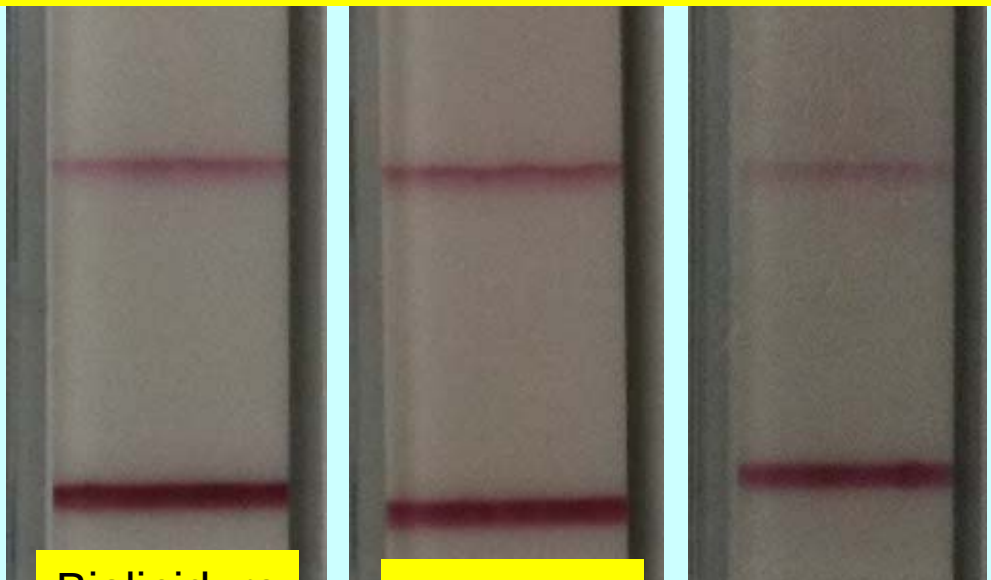
2-4-2. Application for Immunochromatography

KIT Name：Adenovirus rapid test kits by Mitsubishi (MHW# 21400AMZ00679000)
How to use: 0.5% polymer (10% Biolipidure) is added into the solution for virus extraction.

Sensitivities are improved by Biolipidure.

Test Line

Control Line

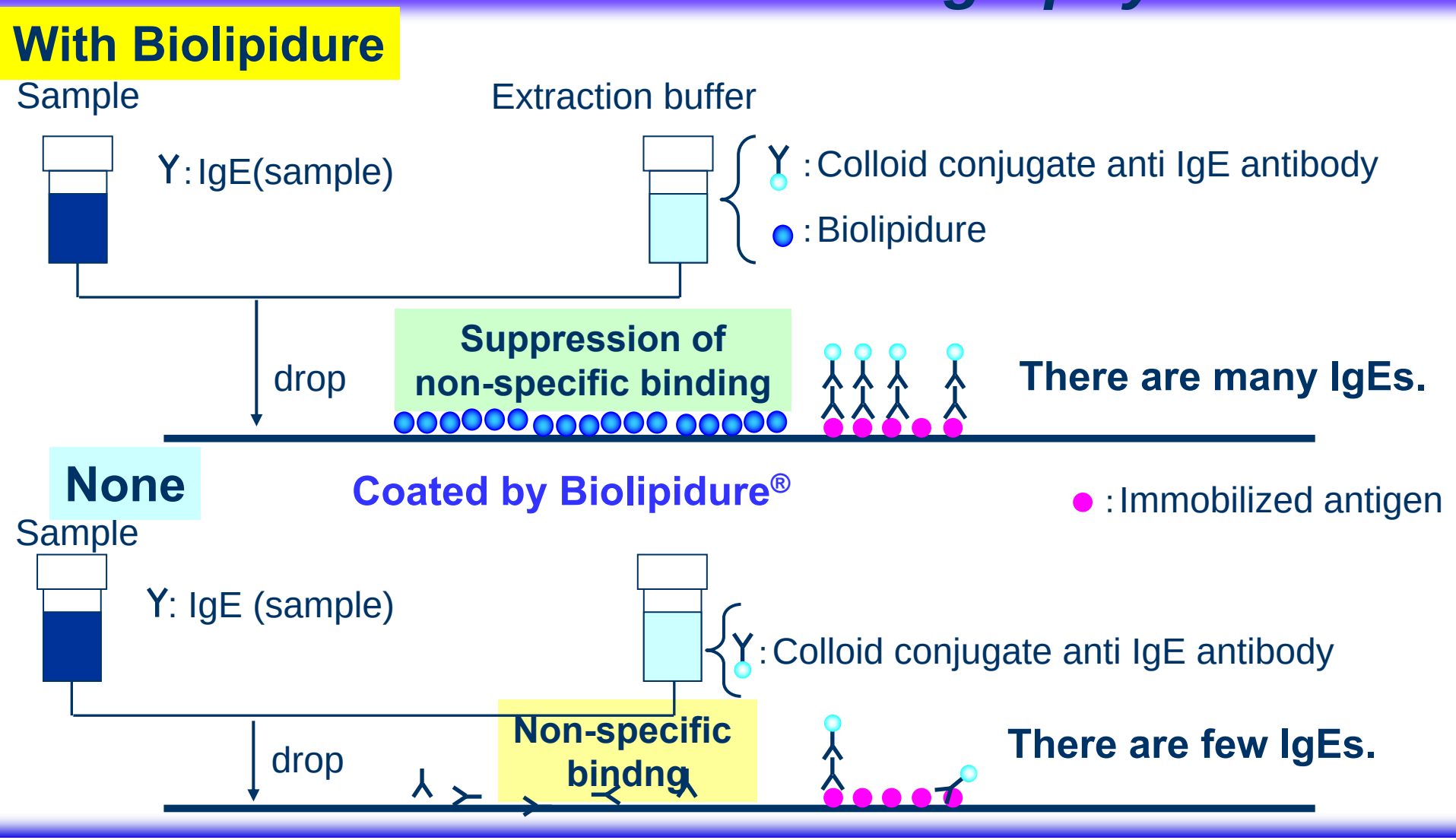


Biolipidure 1301

Biolipidure 203

None

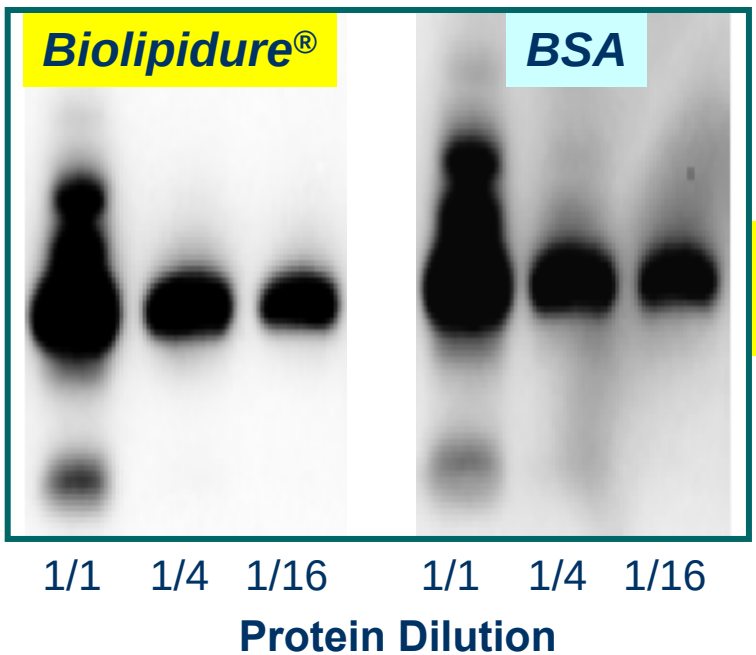
Suppression of Non Specific Binding for immunochromatography



2-4-3. Application for Western Blotting

How to use

1) The proteins are electrophoresed on a 10% SDS-PAGE.
2) The proteins are transferred onto a nitrocellulose membrane.
3) The membrane is blocked with 0.2% polymer (4% “Biolipidure®-203”) in Tris-buffered saline (TBS) at room temperature for 1 hr.
4) The membrane is incubated with appropriate primary antibodies in TBS.



- **Suppress Non-Specific Binding**
- **Improve S/N ratio**

3. Products Line of Biolipidure®

Name	Property	Blocking	Stabilizer	Enhancer	Vol.
Biolipidure®-103	Amphoteric	_	Good	3 *(2)	10 mL
Biolipidure®-203	Amphoteric	Good	Good	2 *(1)	10 mL
Biolipidure®-206	Amphoteric	2	2	Good	10 mL
Biolipidure®-405	Anionic	_	_	1 *(2)	10 mL
Biolipidure®-502	Cationic	_	_	Good	10 mL
Biolipidure®-702	Amphoteric	_	_	Good	10 mL
Biolipidure®-802	Amphoteric	1	1	_	10 mL
Biolipidure®-1002	Amphoteric	3	Good	Good	10 mL
Biolipidure®-1201	Amphoteric	Good	_	_	10 mL
Biolipidure®-1301	Amphoteric	Good	_	_	10 mL

 # : Immunoassay ***(#) : Latex aggregation method**

4. Summary

- **Biolipidure® performs better than BSA and has no lot-to-lot variations.**
- **Biolipidure® suppresses Non-Specific Binding and improves sensitivity.**
- **Biolipidure® stabilizes IgG on the well and in the buffer solution.**
- **You can chose appropriate Biolipidure®.**