Latex-in-a-Box™ Conjugation Kit for preparing highly reactive antibody (purified) and protein (purified and soluble) latex-particle conjugates

Produce Code: LIB03-B007 – (0.3 µm Blue Latex)

Introduction

Lateral flow chromatographic and flow-through tests offer fast detection of critical components for use in point-of-care testing. The key to these tests is the ability to covalently attach antibodies/proteins to intensely colored nanometer particles. Latex particles that bind ligands through a carboxyl bond or via passive absorption (hydrophobically) have proven highly successful for this application. For optimal binding of the antibody/protein while retaining a high degree of specific activity, the pH of the latex suspension must be adjusted to slightly above the isoelectric point of the coating antibody/protein. This is done through a series of pH titrations with the provided buffers. Varying amounts of buffers A and B, and varying amounts of buffers C and D are added to the latex particles to create a pH 5.4 - 10.1 range. Next, antibody/protein is added, and after 30 minutes, the reaction is stopped. After a wash step, the particles are ready for testing. This convenient **Latex-in-a-Box™** kit allows you to quickly (in less than an hour) determine the pI and optimal coating range for your antibody or soluble protein.

Kit Components

1. LP03-B007 – 2.5% **BLUE** 0.3 micron latex particles - 7 mL (white cap)
2. BUFA-001 – Buffer Solution A - 1.0 mL (cap with **black** dot)
3. BUFB-001 – Buffer Solution B - 1.0 mL (cap with **green** dot)
4. BUFC-001 – Buffer Solution C - 1.0 mL (cap with **blue** dot)
5. BUFD-001 – Buffer Solution D - 1.0 mL (cap with **red** dot)
6. BLK-002 – BSA Blocking Stabilizer Solution - 2.0 mL (clear cap)
7. CDB-002 – Latex Conjugate Drying Buffer – 2 mL (cap with **purple** dot)

Materials/Equipment Required but Not Provided

1. Test Tubes (12 x 75 mm)
2. Pipettes and Tips
3. Laboratory Grade Water – Type 2
4. 0.5X PBS
5. 1X PBS
6. Storage Buffer (1X PBS, 0.1% BSA and 0.05% ProClin® 950)
7. Vortexer
8. Centrifuge
9. Polyester or Glass Fiber Ribbon
10. Vented Incubator or Source of Clean and Dry, Flowing Warm Air

Materials Required but Not Provided for Covalent Coupling Procedure

1. 0.1 M MES Buffer, pH 5.0
   [M 2-(N-Morpholino) ethanesulfonic acid]
2. Water Soluble Carbodiimide
   a. 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) **or**
   b. 1-cyclonhexyl-3(2-morphonlinoethyl) carbodiimide metho-p toluensulfonate (CMC)

Sample Preparation

The antibodies/proteins used with this kit must be at a concentration of 1 mg/mL or greater.

For passive conjugation, antibody/protein should be reconstituted with or dialyzed against a 0.5X PBS buffer solution. Antibodies/proteins at a concentration of 2 mg/mL or greater should be in 1X PBS.

For covalent conjugation, antibody/protein should be reconstituted with or dialyzed against 0.1 M MES buffer, pH 5.0.

Researchers are advised to optimize the protein to latex ratio and incubation times for their specific antibody/protein.

Note: Use aseptic technique when handling the latex particles.

Generic Passive Coating Procedure

Helpful Hint

If you know the isoelectric point of your antibody/protein, choose the buffer conditions from the pH Chart on Page 2 at and slightly higher than the isoelectric point (e.g., if the pI of your antibody/protein is 7.3, choose buffer conditions for pH's 7.3, 7.8, and 8.2).

1. Shake or swirl the latex particles (LP03-B007) to re-suspend any settled latex microspheres. Place 0.5 mL of the latex particles into each of ten (10) test tubes (or into the number of test tubes desired at and slightly higher than your antibody/protein's pI).
2. Label each test tube with the pH value (or Tube 1 through 10) from the pH Chart provided on Page 2.
3. Add Buffer Solutions as specified in the pH Chart on Page 2 to each test tube. Shake/vortex to mix.
4. Place each test tube on a low speed vortexer and add antibody/protein solution (See "Sample Preparation Section") - mix thoroughly (about 3 to 5 seconds).

Typically, for the 300 nm latex particles, 250 µL of a 1 mg/mL solution of antibody/protein is optimal.

Note: The saturation point for 300 nm latex particles is usually about 500 µg of antibody/protein per mL of latex particles.

5. Allow the reaction to continue for 30 minutes. Longer incubation times may result in greater latex–antibody/protein binding.
6. Add 50 µL of BSA Blocking Stabilizer Solution (BLK-002) to each test tube and mix thoroughly.
7. Add 2 mL of 1X PBS to each test tube and mix thoroughly.
8. Centrifuge the solution at 1000 × G for 10 minutes to wash.
9. Remove the supernatant fluid and vortex the pellet.
10. Add 2 mL of 1X PBS to the pellet and vortex to re-suspend.
11. Centrifuge the solution at 1000 × G for 10 minutes to wash.
12. Remove the supernatant fluid and vortex the pellet.
13. Re-suspend the latex conjugate solution in 0.5 mL of Storage Buffer (1X PBS, 0.1% BSA, and 0.05% ProClin® 950) and store refrigerated, 2-8°C.

Note: Cross-linking of the latex often causes an aggregation of latex particles. If aggregation occurs, use a bath sonicator to break down any aggregates. Usually, sonication for 10 to 30 seconds is sufficient.

Note: Do no heat the latex solution to greater than 50°C.

See section below on "Stability of Latex Conjugates."

Covalent Coupling of Protein to Latex Particles

1. Add 1.0 mL of 0.1 Molar MES buffer, pH 5.0, to 1.0 mL of latex particles (LP03-B007). Mix thoroughly.
2. Centrifuge the solution at 1000 × G for 10 minutes to wash.
3. Remove the supernatant fluid and vortex the pellet.
4. Re-suspend the latex particle solution in 2 mL MES buffer. Mix thoroughly.
5. Centrifuge the solution at 1000 × G for 10 minutes to wash.
6. Remove the supernatant fluid and vortex the pellet.
7. Re-suspend the latex particle solution in 0.25 mL MES buffer.
8. Dissolve 10 mg EDC or CMC in 0.25 mL MES buffer.
9. Add the freshly prepared EDC or CMC solution (step 8) to the washed particle solution (step 7) and mix gently end-over-end or vortex briefly. Allow the activation to proceed for 10 minutes.
10. Add antibody/protein (e.g., 500 µg antibody/protein dissolved in 0.1M MES buffer) to the activated particles and mix the suspension gently for one hour (60 minutes) at room temperature (20-25°C). End-over-end mixing is best. Longer incubation times may result in greater latex–antibody/protein binding.

Typically, for the 300 nm latex particles, 500 µL of a 1 mg/mL solution of antibody/protein is optimal.

BioAssay Works®, LLC • 10075 Tyler Place • Suite #18 • Ijamsville, MD 21754 • U.S.A. • (301) 874-8888 • FAX: (301) 874-8889

Note: The saturation point for 300 nm latex particles is usually about 500 µg of antibody/protein per mL of latex particles.

11. Centrifuge the solution at 1000 × G for 10 minutes to wash.
12. Remove the supernatant fluid and vortex the pellet.
13. Add 2 mL of 1X PBS and mix thoroughly.
14. Centrifuge the solution at 1000 × G for 10 minutes to wash.
15. Remove the supernatant fluid and vortex the pellet.
16. Add 2 mL of 1X PBS and mix thoroughly.
17. Centrifuge the solution at 1000 × G for 10 minutes to wash.
18. Remove the supernatant fluid and vortex the pellet.
19. Re-suspend the latex conjugate solution in 1.0 mL of Storage Buffer (1X PBS, 0.1% BSA, 0.05% Proclin® 950) and store refrigerated, 2-8°C.

Note: Cross-linking of the latex often causes an aggregation of latex particles. If aggregation occurs, use a bath sonicator to break down any aggregates. Usually, sonication for 10 to 30 seconds is sufficient.

Note: Do no heat latex solution to greater than 50°C.

See section below on "Stability of Latex Conjugates."

Stability of Latex Conjugates

Latex particles completely coated with protein take on the properties of the coating proteins and become very stable in solutions of high ionic strength; however, cross-linking of the latex often causes an aggregation of latex particles. If aggregation occurs, use a bath sonicator to break down any aggregates. Usually, sonication for 10 to 30 seconds is sufficient. Do no heat latex solution to greater than 50°C.

In order to effectively dry down the latex conjugate solution, add 0.1 mL of Latex Conjugate Drying Buffer (CDB-002) for every 1.0 mL of latex conjugate solution. Mix thoroughly. Apply gradually and evenly to either glass fiber or polyester ribbon.

Place coated **polyester ribbon** in a vented 37°C oven or incubator for four (4) hours to dry thoroughly. Coated **glass fiber** ribbon should be left in the incubator overnight (16-20 hours) to ensure complete drying.

The following alternative drying procedure is for polyester ribbon only:

If a vented incubator is not available, use a hairdryer or other clean, dry warm air source set to deliver 30-45°C heat at a ten (10) inch distance from the ribbon surface. Usually, 5 to 15 minutes in this direct warm air flow will suffice to thoroughly dry the ribbon.

Store all coated and dried ribbon in a tightly sealable, moisture proof container containing ample desiccants (granular or pouch form).

This generic procedure may be modified or scaled as needed. When developing a new assay/latex conjugate, it is important to determine the optimal quantity of protein to react with the latex particles. Once the conjugates have been assayed, it is useful to further optimize binding by both decreasing and increasing the quantity of antibody/protein added to the reaction. Often, a 20% increase or decrease in antibody/protein added is sufficient to determine an optimal coating procedure. A few cases may require a 40% or more increase or decrease in coating antibody/protein. Incubation times may also be adjusted to optimize the conjugation procedure.

Discussion

A sensitive lateral flow assay requires that all of the antibody/protein that is added to the latex particles be irreversibly bound to the particles. Any free antibody/protein competes with the bound antibody/protein and reduces the sensitivity of the assay. This behavior ultimately sets the sensitivity limits of an assay.

Latex particles remain in solution because they repel each other due to a significant negative charge. Any latex particle or latex conjugate solution should be shaken before use to re-suspend any settled microspheres.

Application of Latex Conjugates

Latex conjugates are excellent for use in a variety of latex conjugate amplified assay procedures. This includes use in the BioAssay Works®' patented ultra-sensitive C-FLAT technology. Researchers interested in evaluating this technology may contact BioAssay Works® for a research use license with no fee.

pH Chart for Optimal Coating at a pH of 5-10 (per 0.5 mL of latex particles)

Tube Number	pH	Buffer A (BUFA-001)	Buffer B (BUFB-001)
1	5.4	9 µL	1 µL
2	6.6	8 µL	2 µL
3	7.3	6 µL	4 µL
4	7.8	4 µL	6 µL
5	8.2	2 µL	8 µL

Tube Number	pH	Buffer C (BUFC-001)	Buffer D (BUFD-001)
6	8.4	10 µL	0 µL
7	8.8	8 µL	2 µL
8	9.2	6 µL	4 µL
9	9.6	4 µL	6 µL
10	10.1	2 µL	8 µL

Please Note:

The BSA Blocking Stabilizer Solution (BLK-002) contains less than 0.1 % ProClin® 950.

ProClin® is corrosive. Direct contact with components that contain ProClin® should be avoided. ProClin® is harmful if inhaled, ingested or absorbed by the skin. Avoid contact with skin, eyes, or clothing. Wear eye or face protection when handling. If skin or eye contact occurs, wash with copious amounts of water. If ingested or inhaled, contact a physician immediately. Refer to the Material Safety Data Sheet for more detailed safety information.

Handling

- Do not eat, drink, smoke or apply cosmetics in laboratory areas.
- Do not mouth pipette reagents or samples.
- Avoid splashing and forming aerosols.
- Follow cGMP/GLP
- Use reagents according to directions in the product insert.

Storage: Store BSA Blocking Stabilizer Solution (BLK-002) at 2-8°C. The other kit components may be stored at room temperature (20-25°C). If the entire kit is stored at 2-8°C, make sure all components warm to room temperature before use.

Store all latex–antibody/protein conjugates produced at 2-8°C.

Do NOT Freeze.

Warranty

BioAssay Works®, LLC • 10075 Tyler Place • Suite #18 • Ijamsville, MD 21754 • U.S.A. • (301) 874-8888 • FAX: (301) 874-8889