**Bead Zoomer™**

**Bead-Based 2-Step Chemiluminescent Sandwich Assay Protocol**

**Assay Principle:**

This assay first mixes the antigen and the beads functionalized with capture antibody together. After desired duration of incubation, the bead mixture is transferred to a Bead Zoomer™ Zoom Plate™, and gets rinsed. Horseradish peroxidase (HRP)-labeled detection antibody is then added onto the beads and binds to the captured antigen. Rinsing buffer is added again to remove the unbound detection antibody. After injecting HRP chemiluminescent substrate, chemiluminescent signal of the beads is detected using a plate reader or a gel imager. The advanced design of Zoom Plate makes it easy to rinse the beads, saving significant amount of time and reagents.

This protocol is provided as a general guideline. Optimal dilutions for the antigen, antibodies, controls, as well as incubation duration and buffer compositions will need to be determined empirically in a specific application.

**Reagents:**

1. Blocking buffer: 10% bovine serum albumin (BSA) in phosphate buffered saline (PBS) with 0.05% Tween (10%BSA-PBST)
2. Rinsing buffer: 1% BSA in PBS with 0.05% Tween (1%BSA-PBST)
3. 5 µm diameter silica beads conjugated with capture antibody, 1% solid stored in rinsing buffer
4. Antigen sample diluted in rinsing buffer
5. HRP conjugated detection antibody, 100 nM in rinsing buffer
6. Enhanced chemiluminescent (ECL) HRP substrate

**Equipment:**

1. Bead Zoomer™ 96 well Zoom Plate™ (cat# BGPF27B)
2. Multichannel pipette
3. Chemiluminescence plate reader or gel imager

**Procedure:**

Before the assay, remove the Bead Zoomer plate from the ziplock pouch, place the strips not to be used back into the pouch and seal the pouch. Make sure all the absorbing plugs are tightly inserted at the bottom of the strips. You can push the plugs into the wells if necessary.
After each injection, make sure all solutions get absorbed completely. If there is a bubble trapped at the reaction zone, use a 20 µl pipette to suck it out.

The total amount of solution added into each well should not exceed the maximum absorbing capacity of the well, which is 350 µl. If more solution needs to be added, you can place an absorbing pad under the plate.

<table>
<thead>
<tr>
<th>Step 1a. Mix 10 µl beads and 10 µl antigen in a microcentrifuge tube. Incubate 30 min or as long as needed.</th>
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<tr>
<td><strong>Step 1b.</strong> Block each Zoom Plate wells with 50 µl blocking buffer and incubate for 20-30 min.</td>
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<td>Note: This step can be done concurrently with Step 1.</td>
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<tr>
<td>Inject blocking buffer at the center of the reaction zone. Pipette tip should be close to the membrane but do not touch the membrane.</td>
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<td>Do not wait for more than 40 min, otherwise the membrane will get dried.</td>
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<td><strong>Step 2.</strong> Add 20 µl bead mixture into each well.</td>
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<td>Note: Inject at the center of the reaction zone. Pipette tip should be close to the membrane but do not touch the membrane.</td>
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<td><strong>Step 3.</strong> Rinse with 50 µl rinsing buffer 2 times.</td>
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<td>Note: When injecting, rest the pipette tip against the wall near the reaction zone. Do not pipette over the reaction zone as this is easy to introduce bubble.</td>
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**Step 4.** Add 1 µl HRP-conjugated detection antibody onto the beads. Incubate 5 min.

   Note: Inject at the center of the reaction zone. Pipette tip should be close to the membrane but do not touch the membrane.

   Do not incubate more than 10 min.

**Step 5.** Rinse with 50 µl rinsing buffer 4 times.

   Note: **When injecting, rest the pipette tip against the wall near the reaction zone.** Do not pipette over the reaction zone as this is easy to introduce bubble.

   Optional: you can rinse with 40 µl rinsing buffer for 5 times. If using a robot, try continuous dripping a total of 200 µl rinsing buffer.

**Step 6.** Add 10 µl HRP substrate, wait for 0-10 min and detect chemiluminescent signal immediately in a plate reader or imaging system.

   Note: Pre-mix ECL substrate components if it is a two-part system.

   Inject at the center of the reaction zone. Pipette tip should be close to the membrane but do not touch the membrane.

   Detect luminescent signal as soon as possible. Do not wait for more than 10 min before detection, as the chemiluminescent signal will change over time.

   If needed, additional rinsing buffer (10 µl) and HRP substrate (10 µl) can be added and signal can be detected again.