INSTRUCTIONS

Easy-Titer® ELIFA System

77000

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<th>Number</th>
<th>Description</th>
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<td>77000</td>
<td>Easy-Titer® ELIFA System</td>
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System Contents:
- Sample application plate
- Top gasket
- Membrane support plate with bottom gasket and 96 cannulas
- Vacuum chamber with o-ring
- Thumbscrews, 4 each
- One-way valves, 4 each
- Valve/tubing adapters, 4 each
- Extra cannulas, 10 each
- Tubing, 4 ft., 1/8" ID; 3/16" OD; 1/32" inner wall

Introduction
The Easy-Titer® ELIFA System enables fast ELISA results by eliminating long incubations required for reactants to diffuse to the reaction surface. The enzyme-linked immunoflow assay (ELIFA) method uses a nitrocellulose membrane sandwiched between a 96-well sample application plate and a vacuum chamber. Reactants are added to the sample application plate and the vacuum pulls reactants through the membrane. Cannulas transfer nonbound products to the collection chamber. For detection, a microplate is placed in the collection chamber before adding the enzyme substrate. The vacuum allows transference of the colored product into microplate wells for analysis in an automated microplate reader (Figure 1).

The Easy-Titer® ELIFA System is ideal for ELISA-type procedures and dot blots. The system is optimized for uniform, slow solution flow rate and pull-through times and precise and quantitative transfer of colored product. ELISA-type procedures can be performed in 25 minutes, reducing one-hour incubations to five minutes. This method is highly sensitive and the tight seal formed by the gaskets eliminates cross-contamination between samples.

Additional Equipment Required
- Variable speed peristaltic pump
- 8 × 12 cm precut nitrocellulose membrane (see the Related Pierce Products Section)

Figure 1. The ELIFA system is composed of precision cut plexiglass with tight sealing gaskets that provide constant flow rates from well to well. The cannulas precisely transfer colored product to microplate wells for analysis.

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Example Procedure

The following protocol is an example application for the Easy-Titer® ELIFA System. Specific applications and systems require optimization. For dot blot procedures, use a precipitating substrate. For ELIFA procedures, use a soluble substrate. (See the Related Pierce Products Section.)

Notes:

- To avoid air bubbles, pipette slowly and carefully. To burst any bubbles, insert a pipette tip into the well and circle the bottom being careful to not damage the membrane.
- When assembling the system or placing a microplate in the collection chamber, open the valve so that solution is not pulled into the wells. For even pull-through, samples should contain equal volumes of solution.
- When adding sample to the wells, keep the valve closed so that gravity does not pull the samples through the cannulas.
- After each pull-through, open the valve to release the vacuum and close the valve before adding the next solution.
- After frequent use the clamps may need adjusting. To adjust clamps, loosen the screw holding the clamp in place and then reposition the clamp to ensure a tight fit.

A. System Preparation

1. Remove the piece of plastic placed between the top gasket and the cannulas.
2. Clamp together the collection chamber and the membrane support plate with the bottom gasket and cannulas in place. Align the top gasket over the cannulas. The upper left corners of all key pieces are notched for proper orientation.
3. Wet a piece of nitrocellulose by placing a precut sheet (8 × 12 cm) in ultrapure water. Wet the entire membrane or uneven flow may occur.
4. Place the wetted nitrocellulose over the top gasket covering all 96 wells. For proper sealing, place the nitrocellulose above both silicone gaskets and not between them.
5. Position the sample application plate using the guide pins attached to the membrane support plate.
6. Insert the four thumbscrews and lightly tighten two of them at diagonal corners. Alternate to the other two thumbscrews and tighten them to a slightly greater degree. Continue this alternating and tightening action until the plates contact the position stops (acrylic balls located on top of the membrane support plate).
7. Close the vacuum relief valve.
8. Attach silicone tubing to the vacuum valve and the other end to a peristaltic pump.
9. Add 100 µl of water to all 96 wells, being careful not to introduce any bubbles into the wells. Turn on the peristaltic pump at the pump's fastest flow rate. If all the wells have an even flow rate, turn off the pump, open the vacuum relief valve to release vacuum and close it again.

B. Test the Flow Rate

Note: Before each assay, check the flow rate to make sure the unit is sealed properly.

1. Assemble the unit and add 100 µl of distilled water to all wells.
2. Pull through with a fast speed. Make sure all samples are pulled through the wells. Turn off the pump and open and close the vacuum release valve.
3. Add 100 µl of distilled water to the wells. Pull through using a peristaltic pump speed to give a flow rate of 100 µl/1.5 minutes/well.
4. Begin timing when the pump is turned on. Note the time interval between the evacuation of the first and the last sample. If the noted time interval is > 30 seconds, then the unit is not getting a good seal. Dismantle and reassemble the unit to ensure a proper seal.

C. ELIFA/Dot Blot Procedure

1. Check that the vacuum is released by opening the vacuum relief valve, and then close the valve.
2. Add 200 µl of a 50 µg/ml antigen solution to all wells.
3. Set the peristaltic pump on a speed that produces a 5 minute flow rate for the total 19.2 ml in the wells and pull through in 5 minutes. Open and close the vacuum relief valve.

4. Block with 200 µl of a 3% BSA, and pull through in 5 minutes. Open and close the vacuum relief valve.

5. Add 200 µl of serial dilutions (standards) and unknown amounts of the primary antibody and pull through in 5 minutes. Open and close the vacuum relief valve.

6. Add 200 µl of a 1:1,000 dilution of the labeled secondary antibody and pull through in 5 minutes. Open and close the vacuum relief valve.

7. To clean any secondary antibody from the cannulas, wash the membrane three times with 200 µl of a buffered solution pulled through in 30 seconds.

8. Open the vacuum relief valve and undo the clamps between the collection chamber and the membrane support plate. Place the top assembly (i.e., sample application plate, nitrocellulose, gasket with cannulas, and membrane support plate) on a paper towel to remove excess liquid from the bottom of the cannulas. Place a flat-bottomed microplate in the collection chamber.

9. Reassemble the unit by clamping the collection chamber and the membrane support plate together so that the cannulas extend into the wells of the microplate. Close the vacuum relief valve.

10. Add 200 µl of a 0.5 mg/ml substrate solution to the wells and pull through in 5 minutes, collecting it in the microplate. Measure the absorbance of each well in an ELISA plate reader.

   **Note:** If the product is too dark, increase the pump flow rate, or if it is too light decrease the flow rate. Repeat the process of adding the substrate solution to obtain the desired intensity.

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### D. Clean the Easy-Titer® ELIFA System

1. Clean all pieces of the unit with a 2% PCC-54® Detergent (Product No. 72288) solution and then rinse with deionized water. To remove stains caused by the substrate, soak the unit in PCC-54® Detergent.

### Troubleshooting

<table>
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<tr>
<th>Problem</th>
<th>Possible Cause</th>
<th>Solution</th>
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<tbody>
<tr>
<td>Background signal</td>
<td>Solution contains precipitate</td>
<td>Filter solution using a 0.2 µm filter or centrifuge at 13,000 ( \times ) ( g ) for 10 minutes.</td>
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<td>Free enzyme remains on the nitrocellulose and in the cannulas</td>
<td>Wash at least three times with ultrapure water or buffer using a volume equal to or greater than the enzyme solution</td>
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<td>Low quality bovine serum albumin (BSA) causes nonspecific binding</td>
<td>Use high quality BSA such as fraction V BSA</td>
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<td>Nonspecific binding to unblocked sites</td>
<td>Prepare the primary or secondary antibody in 1% normal serum from the same species</td>
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<td>Highly concentrated solution clogs the membrane</td>
<td>Use a greater volume of a more dilute solution through the wells</td>
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<td>Pull-through interval is too long</td>
<td>Protein is clogging the nitrocellulose</td>
<td>Avoid proteins that can clog the nitrocellulose, such as gelatin, and detergents such as Tween®-20 or use a membrane with a larger pore size</td>
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<td>Vacuum leak</td>
<td>Check that the O-ring gasket and nitrocellulose are in place and the thumb screws are screwed down so sample application plate in contact with the compression stops (acrylic balls) of the support plate</td>
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<td>Bubble in well</td>
<td>Remove bubble with a pipette tip by circling it around the inside of the well being careful not to damage the nitrocellulose</td>
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| Coefficient of variation (CV) is too high | Solution pulled through too slowly (≥ 20 minutes) may cause well-to-well variation | Increase speed of pull-through to 5 minutes. |
Related Pierce Products

77010  0.45 µm Nitrocellulose, 25/pkg
77012  0.2 µm Nitrocellulose, 25/pkg
72288  PCC-54® Detergent Concentrate, 3 L
72289  PCC-54® Detergent Concentrate, 4 × 3 L
34042  1-Step™ NBT/BCIP, 250 ml, precipitating substrate for alkaline phosphatase
37621  1-Step™ PNPP, 100 ml, soluble substrate for alkaline phosphatase
34065  Metal Enhanced DAB Substrate Kit, precipitating substrate for horseradish peroxidase
34028  1-Step™ Ultra TMB-ELISA, soluble substrate for horseradish peroxidase
28372  BupH™ Phosphate Buffered Saline Packs, 40 packs
28379  BupH™ Tris Buffered Saline Packs, 40 packs

References


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Current versions of product instructions are available at www.piercenet.com. For a faxed copy, call 800-874-3723 or contact your local distributor.