



Instructions for ImmunoBooster™ ELISA

Important Product Information

- The stability of primary antibodies diluted in the ImmunoBooster ELISA Antibody Diluent varies. For best results, dilute the antibody in the working solution no more than 24 hours before use. However most antibodies are stable up to four weeks in ImmunoBooster if stored at +4°C.
- It is recommended as good laboratory practice to use gloves and personal protective gears, especially as serum samples used in ELISA assays may be infectious.

Material Included

- **ImmunoBooster ELISA Antibody Diluent** (ready solution). **Catalog #: IBE-25 or IBE-50**

Additional Materials Required

- ELISA plates coated with antigen or capture antibody.
- PBS or TBS containing 0.05% Tween-20
- Primary Antibody: Choose a mouse, rabbit or human antibody that is specific to the target protein(s). The optimal dilution to use depends on the specific primary antibody and the amount of antigen on the membrane. However, in our experience the same dilution which was used with the traditional ELISA works well. *In most cases, the primary antibody can be diluted 2 to 4 fold more with comparable results to the traditional ELISA.*

Procedure for indirect ELISA

1. Dilute your primary antibody in ImmunoBooster ELISA Antibody Diluent according to dilutions used in regular ELISA method.
2. Add 100 µl of diluted antibody to each well and incubate for 10 minutes at RT (incubation time can be extended to no more than 15 minutes)
3. Wash the wells four times with PBS or TBS containing 0.05% Tween-20.
4. Immediately add 100 µl of Secondary Antibody or streptavidin, diluted in the ImmunoBooster ELISA Antibody Diluent to each well and incubate for 10 minutes at RT.
5. Wash the wells four times with PBS or TBS containing 0.05% Tween-20.
6. Add 100 µl of substrate to each well and develop according to your standard method.

Procedure for capture ELISA

1. Dilute your antigen containing material in ImmunoBooster ELISA Antibody Diluent according to dilutions used in regular ELISA method.
2. Add 100 µl of diluted antigen to each well and incubate for 10 minutes at RT (incubation time can be extended to no more than 15 minutes)
3. Wash the wells four times with PBS or TBS containing 0.05% Tween-20.
4. Immediately add 100 µl of Detection Antibody diluted in the ImmunoBooster ELISA Antibody Diluent to each well and incubate for 10 minutes at RT.
5. Wash the wells four times with PBS or TBS containing 0.05% Tween-20.
6. Immediately add 100 µl of your labeled antibody or streptavidin diluted in ImmunoBooster ELISA Antibody Diluent to each well and incubate for 10 minutes at RT.
7. Wash the wells four times with PBS or TBS containing 0.05% Tween-20.
8. Add 100 µl of substrate to each well and develop according to your standard method.

Primary and secondary incubation times tested:

Below is the result of various incubation times using a primary antibody (mouse monoclonal) at various concentration and the secondary anti-mouse IgG-HRP at 1/10,000 dilution.

15+ 5 min = 15 minutes with primary antibody and 5 minutes with secondary antibody

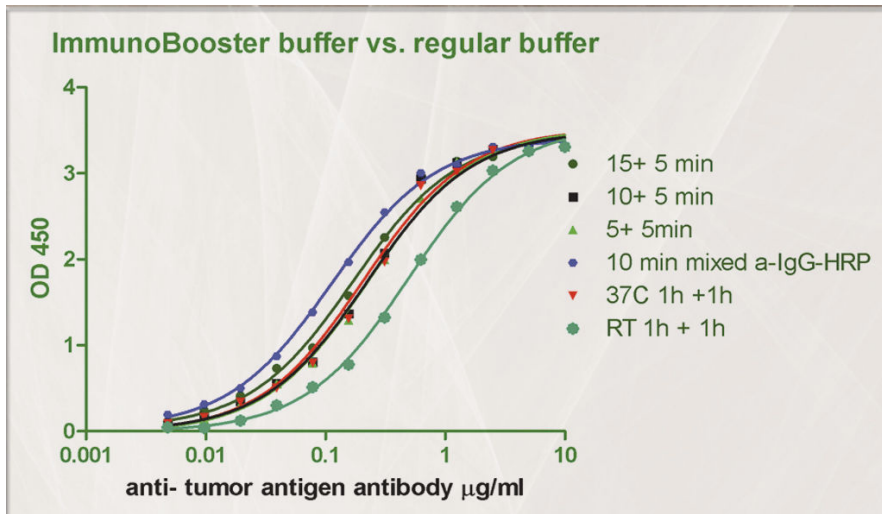
10+ 5 min = 10 minutes with primary antibody and 5 minutes with secondary antibody

5+ 5 min = 5 minutes with primary antibody and 5 minutes with secondary antibody

10 min mixed a- IgG-HRP = primary and secondary antibody was premixed, added to wells and incubated for 10 minutes (**one step**)

37C 1h + 1h = Traditional 1 hour incubation with primary antibody then 1 hour with secondary antibody at 37°C

RT 1h + 1h = Traditional 1 hour incubation with primary antibody then 1 hour with secondary antibody at RT



ELISA plate was coated with a colon cancer tumor antigen and the anti-CA antibody (serially diluted) was titrated using regular buffer and 1 hour incubation at room temperature (RT) or 37C (37C). ImmunoBooster was tested at room temperature and various incubation times between 5 and 15 minutes.

Quality control:

Each lot of ImmunoBooster for ELISA is tested according to protocol above. Released lot should give at least same or better EC50 value than the control at 37°C.

Traditional ELISA EC50 at 37°C = 0.0490

ImmunoBooster EC50 at 10+ 5 min = 0.0416

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