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Instructions for ImmunoBooster™ ELISA

Introduction

The BIOWORLD Consulting Laboratories ImmunoBooster ELISA buffer contains optimized reagents that shorten the time to perform a typical ELISA from 2 to 4 hours to ~20 minutes (Patent Pending). The buffer can be used with mouse, rabbit or human primary antibody. Any secondary antibody labeled with HRP, alk. Phosphatase, biotin, or with any type of fluorescence labels can be used. The ImmunoBooster buffer works in direct, indirect, capture and competitive ELISA.
The protocol requires minimal hands-on time and yields results comparable or better than the regular ELISA assay.
This protocol available for download at http:// www.bioworldantibodies.com/info/ELISA.pdf

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 stabile up to four weeks in ImmunoBoster if stored at $+4^0$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.

One Step ELISA

Important Information

One step ELISA only works with monoclonal primary antibodies; do not use it with polyclonal primary antibodies. It also works with Streptavidin-HRP if your primary antibodies are biotin labeled.

The secondary antibody must be **anti-IgG Fc** it cannot be anti-IgG H&L (anti-IgG H&L will block the antigen binding site).

Only 50 µl of each reagents will be used therefore your antibody use will be the same as with the two step method, however your ImmunoBooster use will be reduced.

Procedure for One Step ELISA

Procedure for indirect ELISA

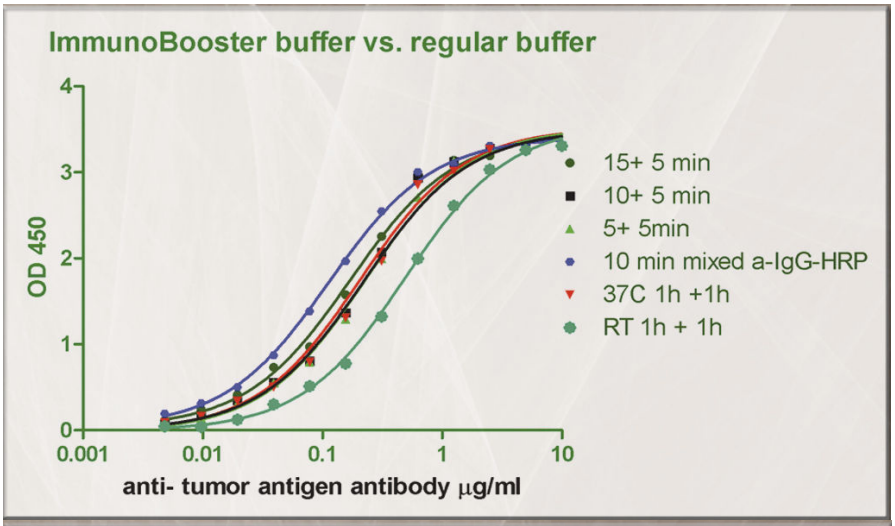
1. Dilute your primary antibody in ImmunoBooster ELISA Antibody Diluent twice to the strength what will be used in regular ELISA method (if it was used 1/2,000 dilute to 1/1,000).
2. Add 50 µl of diluted antibody to each well.
3. Dilute your secondary antibody (anti-IgG Fc) in ImmunoBooster ELISA Antibody Diluent twice to strength what will be used in regular ELISA method (if it was used 1/10,000 dilute to 1/5,000.
4. Add 50 µl of diluted antibody to each well.
5. Add 50 µl of diluted secondary antibody to each well.
6. Mix by shaking the plate (The two reagents can also be premixed in a 96-well dilution plate or tubes, and then transferred to the ELISA plate using multichannel pipet).
7. Incubate the plate for 10 minutes at RT (incubation time can be extended to 15 minutes).
8. Wash the wells four times with PBS or TBS containing 0.05% Tween-20.
9. 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 Fc-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

For one step ELISA the secondary antibody most be anti-IgG Fc



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 beween 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

Date: 04/27/2012