Enzyme Linked immunosorbent Assay (ELISA) for Soluble and Cellular Antigens

The main principle of the ELISA technique is that in the final step of the assay, the anti-immunoglobulin or protein A is enzyme labeled instead of radioiodinated. The degree of antibody binding is evaluated by color development that follows the addition of substrate to the system. The amount of color developed is proportional to the level of enzyme-bound antibody present.
Although ELISA is somewhat less sensitive than RIA, the handling of samples, color development and its measurement are faster, especially with the automatic scanners that enable the reading, typing, and computing of the results of 96 wells within 1-2 minutes.
Another practical advantage of ELISA, especially for the screening procedure, is that it allows fast evaluation of results and selection of positive wells based allows fast evaluation of results and selection of positive wells based on visual observation.
Here we describe in more detail the procedure of ELISA using peroxidase conjugated anti-Ig, although other enzyme conjugates like alkaline phosphatase and β-galactosidase are available.

Materials:

Antigen: 2-10 µg/ml in PBS.
Plates: ELISA grade, flat bottom or V-shape, non-sterile microtitre plates.
Antibodies: Hybridoma culture supernatant.
PBS/T: PBS containing 0.05% Tween-20.
Enzyme conjugate: peroxidase conjugated to IgG fraction of anti-mouse-Ig (commercially available) diluted in PBS+0.1% BSA+0.05% Tween 20.
Substrate : 2,2'-azinodi-(3-ethylbenzthiazoline sulfonic acid) diammonium salt (ATBS) Sigma A-1888.
Substrate solution: ATBS 1 mg/ml.
H202 0.003% in citrate-phosphate buffer freshly prepared by the mixing of 2.8ml of 0.1M citric acid + 2.2 ml of 0.2M Na2HPO4 +5.0 ml H20 + 100 mg ATBS+ 10µl of 30% H202 .
Stop solution: 0.2M citric acid (may be kept at 4 ºC).

Procedure:

1. Add 100 µl of antigen-solution to each well.
2. Leave plate (covered) for 1-3 hr at RT.
3. Remove unbound antigen and wash wells x 3 with PBS/T.
4. Add 50-100 µl of each antibody dilution or supernatant to each well and incubate at 37ºC for 30 min or 1-2 hr at RT.
5. Wash plate three times with PBS/T.
6. Add to each well 200 µl of the anti-Ig-peroxidase conjugate diluted freshly in PBS/T.

Use conjugate dilutions that are recommended by the manufacturer, or determine the dilution in preliminary titration.
7. Incubate at 37ºC for 30 min or 1-2 hr at RT.
8. Wash plate x 4 with PBS/T.
9. Add 100 µl of freshly made substrate solution, incubate at RT and watch color development (usually within 30 min).
10. Add 100µl of 0.2M citric acid to each well, to stop the reaction.
11. Read results in the automatic microelisa reader, using 630 nm filter.

Notes:

a. Avoid use of NaN3 after step 4, since it inhibits peroxidase activity
b. Some cells contain high endogenous levels of enzymes which might result in high background, for example, B lymphocytes contain alkaline phosphatase and macrophages contain peroxidase, therefore, if these cells are serving as target antigen, it is preferable to use another enzyme like β-galactosidase or urease.
c. For accurate reading, avoid scratches on the plate bottoms.
d. some automatic scanners (like the Dynatech Multiscan, for example) can accurately monitor the cheaper, flexible "u" or "v" shape PVC microtitre plates.
e. Nunc (Denemark) has developed a very helpful system, (Nunc TSP) in which the microtiter plate lid has 96 sterile plastic prongs that fit the wells of the microculture plate.

These can be sterilely coated with antigen, dipped into the hybrid culture plate, left to let the antibody bind, removed, washed and processed for ELISA in the assay plate.