Technical Information

Recommendations

The following information, comments and recommendations should give you answers to frequently asked questions and provide some help in obtaining satisfactory results with your ELISA tests.

Test procedure and reagent format: Generally, our ELISA reagents are optimized for use in the double antibody sandwich procedure (DAS-ELISA) on certified Nunc-Immuno Plates MaxiSorp F96 and with a working volume of 200 µl per well. It is described in detail on on the Technical information «ELISA procedure». Other procedures are used (exceptionally) in special cases only.

Outer-row-wells of the microtiter plate: Tight covering of plates (e.g. with parafilm, rubber sheets and covers) and placing them in a moist chamber during incubation help eliminating possible outer-row-effect (edge-effect).

Changing of coating conditions: Alternatively to our standard procedure of 4 h at 30°C, an overnight incubation in the refrigerator at 4 °C is possible. It is also feasible to leave the coating solution for several days in the refrigerator (plates covered, preservative such as NaN_3 in the buffer). Alternatively, you can coat before the test season and keep the washed and emptied plates frozen (-20°C) for several weeks. But repeated freezing/thawing of precoated plates must be avoided. Optionally, a short incubation time such as 2 1/2 h at 37°C gives similar results.

ELISA microtiter plate: **The plate has a dramatic effect on the outcome of the test result.** The plate criterion is not equally obvious for all reagents. Some reagents optimized for one type of plates may perform very poorly on some other plates. **Our reagents are standardized in certified Nunc-Immuno Plates MaxiSorp F96.** The plates are included in our reagent sets and complete kits. Plates can also be ordered separately (see our catalogue or at our homegate in the internet. We strongly urge you to use the same plate for optimal results.

Washing the plate: Careful washing is important. A commercially available ELISA washer, e.g. our EASY WASH 2000, is a possibility. It provides a repeatable and standardized washing. If you have a low number of plates to wash, a squeezing bottle does the job as well. Empty the wells by quickly turning over the plate to prevent overflow into other wells and tap the plate onto absorbent tissue (such as paper towels) before filling the next time with washing buffer. Repeat washing 3-4 times. Please pay special attention to washing out «sticky» plant debris (after antigen incubation) and after conjugate incubation (before adding the substrate). The washing procedure may need adaptation to the washing equipment available in your laboratory.

Drying of test wells between steps: **DO NOT** let a plate stand with empty wells (even not for taking a telephone call), since this may considerably reduce the test performance (at least with certain antibodies). Either keep the wells filled with washing buffer or place it upside down on a wet towel. During prolonged work on a plate (e.g. while working simultaneously at multiple plates), a partial covering of the plate can prevent loss of activity (and irregular results). Determine the loading pattern before starting the test.

Special extraction buffer/sample preparation: Depending on the plant samples to be analyzed, different extraction buffers are required. For most plants, the extraction buffer «General » is adequate. However, for testing grapevines, the extraction buffer «Grapevine» is used. For testing tubers and sprouts of potatoes, bulbs of onion and other Allium spp. as well as for seeds of lettuce (for LMV tests), the extraction buffer «Bulbs and Tubers» is recommended. It consists of the extraction buffer «General» to which egg albumin (ovalbumin) is added. This considerably reduces non-specific reactions induced by some plant tissues. For all tests that need other extraction buffers than the «General», you will find the information on the datasheet delivered with the reagents. All buffers should be stored at 4 °C, but used at room temperature (20-25°C). Thus, bring the aliquot needed for the actual test to room temperature before use.

OD values (extinction values): Most of our tests employ pNPP as the substrate. The color development is measured at 405 nm. If your reader is equipped with dual filters, we suggest reading at 405/492 nm. Reading with dual filters reduces «irregular background» compared to single filter reading at 405 nm. OD values may vary according to species, variety, tissue, physiological age of test plants, pathogen strain, microtiter plate, ELISA plate washer, ELISA plate reader and quality of chemicals.

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