PRODUCT INFORMATION

AquaSpark™ Alkaline Phosphatase Substrate Cat. no. 42593

AquaSpark™ Alkaline Phosphatase Substrate is an optimized chemiluminescent substrate for alkaline phosphatase (AP) detection in immunoassays, particularly in ELISA. The substrate is also a suitable substrate for Western blotting or nucleic acid blotting procedures using AP-conjugated antibodies.

Storage: Store AquaSpark™ Alkaline Phosphatase Substrate (stock solution 2 mM in DMSO) at -15 °C to - 25 °C, protected from light.

Working solution

Prepare working solutions in 100 mM Tris-HCl pH 9.7 with 1 mM $MgCl_2$.
Incubate fresh working solution for 30 min at room temperature before use.
This short pre-incubation time in aqueous buffer solution will eliminate background signal caused by free dioxetane molecules.

The working solution of AquaSpark™ phosphatase substrate can be stored at room temperature for several hours and up to one week at 2 °C – 8 °C.

Use AquaSpark™ Alkaline Phosphatase Substrate at a final concentration of 10 µM. For best results optimize the final concentration for your test conditions.

To prepare a 10 µM working solution dilute the substrate stock solution as follows:
For 2 ml of a 10 µM working solution add 10 µl of AquaSpark™ Alkaline Phosphatase Substrate (2 mM in DMSO) to 1,990 µl Tris buffer solution.
Mix well.

100 µl of AquaSpark™ Alkaline Phosphatase Substrate, 2 mM in DMSO give 20 ml of working solution.

Emission and Detection

The maximum emission wavelength of the activated substrate is 508 nm.
Recommended detection wavelength for AquaSpark™ chemiluminescence emission is 500 – 520 nm.

Sandwich ELISA procedure

1. Prepare a surface to which a defined quantity of capture antibody is bound.
2. Block any nonspecific binding sites on the surface.
3. Apply the antigen-containing sample to the plate.
4. Wash the plate, so that unbound antigen is removed.
5. Add a specific antibody that binds to the antigen
6. Apply AP-conjugated secondary antibodies as detection antibodies that bind specifically to the antibody's Fc region (non-specific).
7. Wash the plate to remove unbound antibody AP-conjugates.
8. Prepare a working solution of AquaSpark™ Alkaline Phosphatase.
   Incubate fresh working solutions for 30 minutes before use. Alternatively, prepare the solution the day before and store it in a fridge over-night.

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Western blot procedure

- Buffer for preparing blocking and washing solution

  TBS-T buffer:
  1x TBS buffer (SERVA cat. no. 42596, 10x concentrate)
  with 0.05 % (v/v) Tween 20 (SERVA cat. no. 37170)

1. Separate proteins by electrophoresis, e.g. SDS PAGE.
2. Electro-transfer proteins from the gel in an appropriate gel transfer apparatus to a membrane (PVDF, nitrocellulose) according to the manufacturer's instructions.
3. Remove the membrane from the transfer apparatus.
   Rinse it briefly with deionized water.
4. Incubate the membrane in blocking solution (mini gel format: 20 ml) for at least 20 - 60 min at RT on a rocking platform, e.g. SERVA BlueShake at RT. As an alternative place the membrane in blocking solution overnight at 4 °C.
5. Immerse the membrane in primary antibody diluted in blocking solution.
   Incubate at least 1 h on a rocking platform, e.g. SERVA BlueShake at RT.
6. Rinse 5 times with 30 ml TBS-T washing solution 5 min per change, draining well after the last wash.
7. Incubate the membrane with an AP-conjugated secondary antibody at the manufacturer's suggested dilution in blocking solution, e.g. 1:5,000 for 30 min on a rocking platform, e.g. SERVA BlueShake at RT.
8. Rinse 5 times with 30 ml TBS-T washing solution 5 min per change.
   Wash longer to minimize background signals.
9. Prepare 5 - 10 ml for one blot of a 10 µM working solution of AquaSpark™ Alkaline Phosphatase Substrate. Incubate fresh working solutions for 30 min before use. Alternatively, prepare the solution the day before and store it in a fridge over-night.
10. Pipette the working solution directly onto the surface of the membrane. Remove excess solution.
11. Measure the luminescence emission in a suitable chemiluminescence imaging system, e.g. SERVA Musketeer or expose the membrane to film.
12. If a film is used for imaging, use an appropriate developing solution and fixative. Light emission of AquaSpark™ is intense. We recommend short exposure times.

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