Product Information

SIGMA*FAST*™ Fast Red TR/Naphthol AS-MX Tablets

Tablet, to prepare 10 mL

F4523

Product Description

Fast Red TR/Naphthol AS-MX is the immunohistology substrate of choice for antibodies conjugated to alkaline phosphatase, as it produces an intense red stain. Slides stained with Fast Red TR/Naphthol AS-MX must be cover-slipped using aqueous mounting media, as the reaction product is alcohol-soluble.

SIGMA*FAST*™ Fast Red TR/Naphthol AS-MX Phosphate (4-Chloro-2-methylbenzenediazonium/ 3-Hydroxy-2-naphthoic acid 2,4-dimethylanilide phosphate) tablets have been developed for use in immunohistology and blotting, as a precipitating substrate for the detection of alkaline phosphatase activity. Levamisole has been added to a concentration of 0.15 mg/mL to block endogenous alkaline phosphatase activity.

SIGMA*FAST*™ Fast Red TR/Naphthol AS-MX tablets require no additional buffers or steps to prepare an active substrate solution. One Fast Red TR/Naphthol AS-MX tablet and one Trizma® buffer tablet, dissolved in 10 mL of deionized or distilled water, provides 10 mL of ready-to-use substrate. Each SIGMA*FAST*™ Fast Red TR/Naphthol AS-MX tablet set contains the following when dissolved in 10 mL H_2O:

- Fast Red TR: 1.0 mg/mL
- Naphthol AS-MX: 0.4 mg/mL
- Levamisole: 0.15 mg/mL
- Trizma® Buffer: 0.1 M

This product has been used to study such systems as animal models,[1-4] flatworms,[5] cultured human cells and tissues,[6,7] and the coeliac immune response.[8] Other references,[9] theses[10-11] and dissertations[12-30] have cited use of F4523 in their research protocols.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store the tablets at –20 °C.

Components

SIGMA*FAST*™ Fast Red TR/Naphthol AS-MX Phosphate Tablets (Component Number F0900): 5 tablets (for 5SET) or 50 tablets (for 50SET)

Trizma® Buffer Tablets (Component Number T1416): 5 tablets (for 5SET) or 50 tablets (for 50SET)

Reagents and Equipment Required but Not Provided

- Distilled or deionized water
- Pipettes
- Test tubes
- 0.2 µm filter (such as Cat. No. WHA10462701)

Preparation Instructions

1. Remove the required number of Fast Red TR/ Naphthol AS-MX and Trizma® tablet packages from the freezer.
2. Allow the tablets to reach room temperature.
3. Open the Trizma® tablet package (gold foil) and drop the tablet into an appropriate container. **Do not touch the tablet with your fingers.**
4. Add 1 mL of distilled or deionized water.
5. Vortex until dissolved.
6. Open the Fast Red TR/Naphthol AS-MX tablet package (silver foil). **Do not touch the tablet with your fingers.**
7. Drop one Fast Red TR/Naphthol AS-MX tablet into the Trizma® buffer. Vortex until dissolved.

The SIGMA*FAST*™ Fast Red TR/Naphthol AS-MX substrate is now ready for use. For best results, the solution should be used within one hour.

Procedure

1. Cover the tissue section with 0.1-0.2 mL of Fast Red TR/Naphthol AS-MX solution.
2. Fast Red TR/Naphthol AS-MX is a fast-reacting substrate. It should be carefully monitored during the reaction to prevent overdevelopment and high background. Reactions may be stopped by gently washing the slide in water.
3. Occasionally, the Fast Red TR/Naphthol AS-MX solution may be hazy. The haziness may be removed by filtering the Fast Red TR/Naphthol AS-MX solution through a 0.2 µm filter.
4. When finished, dispose of any remaining substrate solution in a manner consistent with proper hazardous material handling protocols for your institution.

Troubleshooting

Background is too high

1. Use a blocking step prior to the application of the primary antibody. Diluted normal serum (10% v/v) from the same species as the secondary antibody generally produces the best results.
2. Decrease the staining time.
3. Titer the conjugate to optimize the working dilution.

No color develops or color is too faint

1. Adjust the concentration of the primary antibody.
2. Adjust the concentration of the secondary antibody.
3. Determine if the enzyme conjugate is active.
4. Consider using an amplifying system such as avidin-biotin.
5. Increase the staining time.
6. Determine if enzymatic treatment (unmasking) of the antigen is required prior to application of the primary antibody.

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F4523dat Rev 03/22 PCS,RBG,GCY,MAM