Monosan® New Fuchsin Kit

REF / Cat. No.: MON-APP182 1 Kit (6ml / 60 Tests)
MON-APP183 1 Kit (125ml / 1250 Tests)

Instructions for use

Intended use
Monosan® New Fuchsin Kit is developed for immunohistochemical and *in situ*-hybridisation staining procedures with alkaline phosphatase. Monosan® New Fuchsin leads to the formation of a magenta-red precipitate at the location of the target antigen or target nucleic acid. The precipitate is insoluble in aqueous and organic solvents and can be observed by light microscopy or fluorescence microscopy (Texas Red filter).
Monosan® New Fuchsin Kit is for research use only, not for drug, diagnostic or other use.

Reagents provided:

REF / Cat. No. MON-APP182

0.5 ml	**New Fuchsin Solution (Chromogen)**	**Reagent 1**
0.5 ml	**New Fuchsin Activator**	**Reagent 2**
6 ml	**Naphthol-Phosphate Buffer**	**Reagent 3**
1	**Dilution Vial**	

REF / Cat. No. MON-APP183

7 ml	**New Fuchsin Solution (Chromogen)**	**Reagent 1**
7 ml	**New Fuchsin Activator**	**Reagent 2**
125 ml	**Naphthol-Phosphate Buffer**	**Reagent 3**
1	**Dilution Vial**	

Storage and Handling
The solutions should be stored at 2-8°C without further dilution. Please store the reagents in a dark place and do not freeze them. Under these conditions the solutions are stable up to the expiry date indicated on the label. Do not use product after the expiry date.
The working solution is stable for about 5 - 10 minutes and should therefore be used directly after preparation. Excess working solution needs to be disposed as hazardous substance.
A positive and a negative control have to be carried out in parallel to the test material. If you observe unusual staining or other deviations from the expected results which could possibly be caused by the kit reagents please contact Monosans' technical support or your local distributor.

Precautions
Use by qualified personnel only.
Some of the reagents could be hazardous for your health. Wear protective clothing to avoid contact of reagents or specimen with eye, skin or mucous membrane. In case of a reagent or specimen coming into contact with a sensitive area, wash the area with large amounts of water.
Microbial contamination of the reagents must be avoided, since otherwise non-specific staining might appear.
Sodium azide (NaN_3), used for stabilisation, is not considered hazardous material in the concentrations used. Sodium azide deposits in drainage pipes made of lead or copper can result in the formation of highly explosive metallic azides. To avoid such deposits in drainage pipes, sodium azide should be discarded in a large volume of running water.
Material safety data sheets (MSDS) are available upon request.

Reagent preparation (preparation of the working solution)
1) Mix 50 µl (1 drop) New Fuchsin Solution (Reagent 1) with 50 µl (1 drop) New Fuchsin Activator (Reagent 2) in the provided Dilution Vial and incubate for about 2 - 10 minutes at room temperature.
2) Add 0.9 ml Naphthol-Phosphate Buffer (Reagent 3) and mix thoroughly.
3) The working solution is stable for about 5 – 10 minutes and should therefore be prepared directly before use.

Staining procedure
1) Rinse the slide with wash buffer after the previous incubation step.
2) Apply the freshly prepared New Fuchsin working solution onto the slide. Incubate for 20 - 30 minutes.
3) Rinse with distilled H_2O.
4) Counterstain with haematoxylin for about 30 seconds up to 5 minutes (depending on the desired staining intensity).
5) Rinse with distilled H_2O.
6) Blueing in tap water for at least 5 minutes.
7) Dehydrate through a graded series of ethanol and clear in xylene. Mount with a permanent mounting medium. Note: It is also possible to mount New Fuchsin with aqueous mounting media.

Quality control
We recommend carrying out a positive and a negative control with every staining run. The positive control permits the validation of appropriate processing of the sample. If the negative control has a positive result, this points to unspecific staining. Please refer to the instructions of the detection system for guidance on general quality control procedures.

Troubleshooting
If you observe unusual staining or other deviations from the expected results please read these instructions carefully, contact Monosans' technical support or your local distributor. Also refer to the instructions of the detection systems for guidance on general troubleshooting.

Expected results
During the reaction of the substrate with alkaline phosphatase in presence of the chromogen New Fuchsin, a magenta-red precipitate is formed at the location of the target antigen or nucleic acid. The precipitate is insoluble in aqueous and organic solvents and can be observed by light or fluorescence microscopy (Texas Red filter).

Limitations of the procedure
Immunohistochemistry is a complex method in which histological as well as immunological detection methods are combined. Tissue processing and handling prior to immunostaining, for example variations in fixation and embedding or the inherent nature of the tissue can cause inconsistent results (Nadji and Morales, 1983).
In some tissues endogenous alkaline phosphatase activity may cause non-specific staining. The enzyme activity can be blocked by adding levamisole to the New Fuchsin working solution. However, neither intestinal nor placental alkaline phosphatase can be blocked with levamisole. Therefore, tissues of this origin should be stained with peroxidase detection systems (i.e. MON-APP116). Background staining due to endogenous biotin can be blocked through an avidin-biotin blocking step prior to the primary antibody incubation step.
A higher sensitivity can be obtained when a second chromogenic substrate step is used (i. e. 2 x 20 min New Fuchsin).
Because of the short stability of the working solution it should be prepared directly before use.
Inadequate counterstaining and mounting can influence the interpretation of the results. A longer exposure to absolute ethanol can result in decreasing staining intensity.
Monosan® guarantees that the product will meet all requirements described from its shipping date until its expiry date, as long as the product is correctly stored and utilized. No additional guarantees can be given. Under no circumstances shall Monosan® be liable for any damages arising out of the use of the reagent provided.

Performance characteristics
Monosan® has conducted studies to evaluate the performance of the kit reagents in combination with standard detection systems. The product has been found to be suitable for the intended use.

Bibliography
Elias JM “Immunohistopathology – A practical Approach to Diagnosis” ASCP Press 2003
Nadji M and Morales AR Ann N.Y. Acad Sci 420:134-9, 1983

FOR RESEARCH USE ONLY, NOT FOR DRUG, DIAGNOSTIC OR OTHER USE.

Explanation of the symbols on the product label:

REF	Bestellnummer Catalog Number Reference du catalogue	LOT	Chargenbezeichnung Batch Code Code du lot	Xi	Reizend Irritant Irritant
Xn	Gesundheitsschädlich Harmful Nocif		Giftig Toxic Toxique		Hersteller / Manufacturer / Fabricant Monosan® Frontstraat 2c 5405 PB Uden The Netherlands Tel: (+31) 413 251115 Fax: (+31) 413 266605 info@monosan.com www.monosan.com
	Verwendbar bis Use By Utiliser jusque				
	Gebrauchsanweisung beachten Consult Instructions for use Consulter les instructions d'utilisation		Lagerungstemperatur Temperature Limitation Limites de température		