General ELISA Reagent Pack 20er

Ref.: Gen-Pack-20

MabTag's **General ELISA Reagent Pack** contains appropriate ELISA material sufficient for processing of 20 microplates (96 wells; 100 µl/well) starting with coating of capture antibody on the microplate until stopping of the enzymatic colour reaction.

MabTag GmbH

!***The customer has just to supply the capture antibody, the detection antibody, HRP-Streptavidin and the standard***!

Content	Storage
20 x 96well-Microplates	room temperature
4x 60 ml Coating-Buffer (ready-to-use)	2-8°C
4 x 250 ml Blocking-Buffer / Reagent-Diluent (ready-to-use)	2-8°C
4 x 250 ml Wash-Buffer (10x concentrated; 100 ml must be filled up to 1L with aqua dest.)	2-8°C
4 x 4 ml TMB-Solution A	2-8°C / protect from light
4 x 60 ml TMB-Solution B (working solution: 10 ml TMB-B + 0.5 ml TMB-A)	2-8°C / protect from light
4 x 30 ml Stop-solution (ready-to-use) ACID → wear gloves & protective glasses	2-8°C

STEP	*INCUBATION*	*PROCEDURE*
Coating Capture-antibody	OVERNIGHT at room temperature	Dilute capture-antibody in COATING-BUFFER sufficiantly. Subsequently transfer 100 µl of this mixture to each well and incubate.
Remove completely capture-antibody by inverting the microplate and blotting it ***vigorously*** against clean paper towels.		
Blocking	1 Hour at room temperature	Add 200 µl BLOCKING-BUFFER to each well and incubate.
Remove BLOCKING-BUFFER completely by inverting the microplate and blotting it ***vigorously*** against clean paper towels.		
Standard & Sample	2 Hours at room temperature	Dilute standard & samples in REAGENT-DILUENT and transfer 100 µl in the respective wells in duplicates. Standard: Make duplicate serial dilutions in REAGENT-DILUENT and begin with a high standard and end with blanks.
Wash 3x ***vigorously*** with WASHING-BUFFER and remove resting buffer completely by inverting the microplate and blotting it ***vigorously*** against clean paper towels.		
Detector-antibody	2 Hours at room temperature	Dilute detector-antibody sufficiantly in REAGENT-DILUENT. Subsequently transfer 100 µl of this working-solution to each well and incubate.
Wash 3x ***vigorously*** with WASHING-BUFFER and remove resting buffer completely by inverting the microplate and blotting it ***vigorously*** against clean paper towels.		
HRP-Streptavidin	30 Min at room temperature	Dilute HRP-Streptavidin sufficiantly in REAGENT-DILUENT. Subsequently transfer 100 µl of this mixture to each well and incubate. (***Note****: this step is unnecessary if the detection-antibody itself is HRP-conjugated*)
Wash 3x ***vigorously*** with WASHING-BUFFER and remove resting buffer completely by inverting the microplate and blotting it ***vigorously*** against clean paper towels.		
TMB substrate	Up to 60 Min* at room temperature **in the dark**	Optionally warm the solution to room temperature before use. Add 100 µl of the TMB-SOLUTION to each well and incubate. Control the development of the colour reaction continuously and stop at an appropriate time point.
Stop solution	-	When the enzymatic colour reaction is sufficiently proceeded stop the reaction by adding of 50 µl stop solution. Read the microplate at **450** nm (if wavelength correction is available, set to 540 nm, 570 nm or 630 nm as reference)

*The speed of enzymatic colour development is influenced by many customer-specific factors. Therefore the incubation time is variable und specific for each test system.

Note:

All incubation steps except Poly-HRP-Streptavidin and TMB substrate could be optionally carried out over-night. Do not use sodium azide-containing solutions, nor add sodium azide to the supplied reagents. Sodium azide inactivates the peroxidase.

Storage:

Specific storage conditions in the table above.

Reconstituted reagents should be stored at -20°C. Please prevent repeated freeze- thaw cycles. Stable for up to 6 months after opening when stored at -20° C. The performance of the unopened reagents is guaranteed until one year after point of delivery.

Precautions for use:

!The stop solution is an acid solution. TMB-Solution A contain H_2O_2 and tetramethylbenzidine (TMB). All Buffers and liquid antibody solutions contain 0.04% (v/v) Proclin®950 as preservative. All these compounds are harmful and cause respiratory, skin and eye irritation. Do not swallow any components of the set/kit (R22). Wear face, eye and hand clothing protection when using this material (S36). Keep out of reach of children (S2). Keep away from food, drink and animal feeding stuff (S13). !These reagents are offered for research purposes only! For *in vitro* use only. MabTag will not be held responsible for patent infringement or other violations that may occur with the use of our products.

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