HISTOPRIME®

CatNo E109

Lot: See Label
Storage: +2 to +8°C
Exp. Date: See Label

HistoGreen

Substrate Kit for Peroxidase

Background	**HistoGreen** is a very sensitive substrate chromogen for use in peroxidase-based immuno-histochemical (IHC) and in situ-hybridisation (ISH) staining procedures. It is perfectly suitable for single as well as for multi staining. The **HistoGreen** substrate kit contains all the reagents necessary to prepare the working solution for application. **HistoGreen** produces with the peroxidase a green reaction product that can be only permanently mounted. It produces with peroxidase and alkaline phosphatase substrates an excellent contrast and is compatible with the counterstains **HistoHematoxylin** and **HistoNuclear Fast Red**. In contrast to DAB **HistoGreen** is neither toxic nor carcinogen.
Kit Contents	Reagents are sufficient for staining at least 1000 tissue sections. 1 dropbottle **HistoGreen Chromogen** (bottle No **1,** 8 ml) 1 bottle **HistoGreen-Puffer** (bottle No **2,** 100 ml) 1 dropbottle H_2O_2 (bottle No **3**, 8 ml) 1 dropbottle (empty) for the working dilution (**G**) For convenience the reagents are supplied in dropper bottles. When dispensing drops, hold the bottle in an inverted vertical position and squeeze gently. To prevent evaporation, secure the opaque caps on the bottle when they are not in use. **DO NOT PIPET REAGENTS DIRECTLY FROM THE BOTTLE.** Proper concentrations of substrate components are assured in preparing the working solution by using the drop dispenser.
Instruction for use	Prepare the **HistoGreen** substrate solution directly before used as follows: Preparation (making of substrate solution) In bottle **G** (working dilution) 2 drops from dropbottle 1 **HistoGreen Chromogen** (bottle No **1**) add to 1 ml **HistoGreen-Buffer** from (bottle No **2**) add to this mixture 2 drops H_2O_2 (bottleNo **3**) and mix well. Rinse the sections before adding the substrate in TBS-buffer (0.05 M TRIS-Base, 0.15 M NaCl, pH 7.2) or PBS by Dulbecco (DPBS) (LINARIS CatNr. GBP1622AL powder, GBF1622YK liquid). Incubate the sections with the substrate solution at room temperature while watching until a sufficient colour intensity is reached. Generally a good staining result should be reached within 1-5 minutes. Wash the stained sections for 2-5 min in TBS or DPBS-buffer and rinse them shortly in aqua. dest. If prefered, the stained section may be counterstained with HistoHematoxylin (LINARIS CatNr EGH3401) or HistoNuclearFast Red (LINARIS CatNr EGH3403). The reaction product of the HistoGreen-Substrate is water-soluble and has to be dehydrated in series of alcohol before mounting and has to be mounted with Vectamount™ (LINARIS CatNr E6003) or similar mounting mediums.
Dehydration	The substrate may fade when kept long in the single alcohol components of the dehydration series. We recommend only 30 sec. per component (better: rinse carefully) and the following dehydration series: Alcohol pure 100% - alcohol pure 100% - alcohol purest 100% - Xylene pure - Xylene pure

LINARIS Biologische Produkte GmbH · Frankenweg 18 · D-69221 Dossenheim
Telefon: ++49(0)621/400677-0 · Telefax: ++49 (0)621/400677-19
E-Mail: info@linaris.de · Internet: http://www.linaris.de · USt!dentNummer DE276264940
Amtsgericht Mannheim, HRB-Nr. 711070 · Geschäftsführer/CEO Dr. Peter HARBARTH, Dr. Steffen ROTH

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Storage	Store **HistoGreen** at 4-8°C and keep it dark (refrigerator). The substrate solution may be prepared and used directly after taking it out of the fridge. Warming up to room temperature is not necessary.
Notes	1. **HistoGreen** should be stored at 4°C and be kept dark. The colour of the staining substrate may get a little dark in course of time without affecting the staining quality of **HistoGreen**. 2. Do not heat up single components or working dilution of the substrate, max. 25°C. 3. Use only ***Xylene of the „pure" quality and do not exceed 30 sec. for every cuvette!*** 4. Do not **mount HistoGreen stained sections in aqueous mounting medias!** 5. Extended incubation times in TBS, DPBS, alcohol or Xylene may reduce the colour intensity. 6. When used in double or multiple staining in combination with other substrates for Peroxidase, **HistoGreen** should be used as the last substrate component for the best results.
Trouble Shooting	1. **The substrate may generate a dark blue reaction product in some cases:** • Impure Xylene or Xylene substitutes are used in the dehydration of the stained sections. *Use only Xylene of the „pure" quality .* • Substrate reaction is too intensive (high expressed antigens): *Dilute the primary antibody and/or the HRP-conjugate strongly and shorten the incubation time of the substrate solution.* • Substrate was heated over 25°C. *Use at room temperature only.* 2. **Substrate fades or merges:** • Sections weren't dehydrated and aqueous mounting medias were used: *Dehydrate stained sections and use only non-aqueous mounting medias.* • Chemical reactions as X-Gal-development were done after the development of **HistoGreen**: *Use and develop **HistoGreen** always as last the component.* • The keeping in the single components of the dehydration was too long: *Use and develop **HistoGreen** always as last the component.*
References	1. U. Kämmerer et al. (2003). Expression of Tumor Markers on Breast and Ovarian Cancer Cell Lines. Anticancer Research 23:1051-1056 2. U. Kämmerer et al. (2003). Immunocompetent cells in the endometrium of fetuses and children. Human Reproduction 18(5):969-975 3. U. Kämmerer et al. (2003). Unique Appearance of Proliferating Antigen-Presenting Cells Expressing DC-SIGN (CD209) in the Decidua of early Human Pregnancy. American Journal of Pathology 162(3):887-896 4. Thomas MA, Lemmer B. (2005). HistoGreen: A new alternative to 3,3'-diaminobenzidine-tetrahydrochloride-dihydrate (DAB) as a peroxidase substrate in immunohistochemistry? Brain Res Brain Res Protoc. 2005 Feb;14 (2):107-18.
Important	*Based on kits components, low toxicity and cancerogenicity could be suspected, but little is known about the toxicity and cancerogenicity of the combination of the substrate components. Care should be taken in the handling and disposing of the reagents.*
E109 040730-2/2	

LINARIS Biologische Produkte GmbH · Frankenweg 18 · D-69221 Dossenheim
Telefon: ++49(0)621/400677-0 · Telefax: ++49 (0)621/400677-19
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