

# IMMUNOLOGICAL SUBSTRATES

Acros Organics offers a high quality range of substrates as ready to use tablets and liquids for the immunological detection of HRP and AP. Quality of water is essential in immunological detection. Acros Organics offers high grade water and a revolutionary support for water free of heavy metals.

## Enzyme: HRP (Horse Radish Peroxidase)

Product	Formulation	Code	Product	Packsize	Suggested application	Colour
TMB	liquid (ready to use)	32805	TMB QUICK	30 ml	ELISA (high sensitivity)	YELLOW (450nm) (with stop reagent)
				100 ml		BLUE (665nm) (no stop reagent)
	liquid (ready to use)	32803	TMB EASY	30 ml	ELISA (high sensitivity) (with stop reagent)	YELLOW (450nm)
				100 ml		BLUE (665nm) (no stop reagent)
OPD	fizzing tablet (containing buffer)	32797	OPD EASY	100x2 mg/tablet	ELISA	YELLOW (492nm) (with stop reagent)
	tablet (without buffer)	32802	OPD EASY	100x10 mg/tablet		
DAB	fizzing tablet (containing buffer pH 7,0)	32800	DAB EASY	50x10 mg /tablet	HISTOCHEMISTRY	BROWN
	fizzing tablet (containing buffer pH 5,0)	32799	DAB EASY	50x10 mg /tablet	BLOTTING	BROWN
AEC	fizzing tablet (containing buffer pH 5,1)	34199	AEC EASY	10x5 mg /tablet	HISTOCHEMISTRY	RED
Urea Peroxide Tablet	fizzing tablet (replaces peroxide addition)	32807	PeroxideEASY	50x5,68 mg/tablet	HRP substrates	

## Enzyme: Alkaline Phosphatase

Product	Formulation	Code	Product	Packsize	Suggested application	Colour
FAST RED	tablet (without buffer)	34207	FAST RED	on request	HISTOCHEMISTRY BLOTTING	RED

### 1. TMB EASY/QUICK substrate

TMB EASY (3,3' tetramethylbenzidine) is an aqueous formulation without any harmful organic solvents for maximized safety and minimized waste.

TMB QUICK (3,3' tetramethylbenzidine) is recommended for very fast assay reactions, giving a higher signal than other standard TMB solutions.

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Казахстан (772)734-952-31

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**How to use:**

*Substrate as well as hydrogen peroxide are included. No other reagents should be added. Dilution is not recommended. Store at 4°C.*

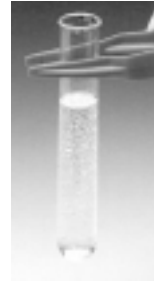
1. *Desired amount of substrate is poured into a sealed container and allowed to reach room temperature in the dark.*
2. *Add 100 µl/microtiterwell substrate solution. Time of development is 5-30 min.*
3. *In kinetic assays the blue color is read at 655 nm. For endpoint assays equal volumes of 0.2 M sulphuric acid is added and the absorbance is read at 450 nm. The obtained yellow solution are stable for 1 hour.*

**2. DAB EASYtablets for immunoblotting/for immunohistochemistry**

DAB EASY (3,3'-diaminobenzidine tetrahydrochloride ) tablets for immunoblotting contain also the citrate buffer giving you the proper pH(5.0) for immunoblotting when dissolved in water.

DAB EASY (3,3'-diaminobenzidine tetrahydrochloride ) tablets for immunohistochemistry contain also the phosphate buffer giving you the proper pH(7.0) for immunohistochemistry when dissolved in water.

Short term storage at 4°C, long term storage at -20°C. Keep the lid tightly closed during storage of the tablets and do not remove the drying capsule from the container.



**How to use:**

1. *Remove the container from the refrigerator and allow to stand at room temperature for 15 minutes. DO NOT USE METAL FORCEPS DURING HANDLING OF THE TABLETS!*
2. *Dissolve the tablet, containing 10 mg DAB, in 10 ml of distilled water at room temperature.*
3. *Some fine particular matter will remain in the suspension even after the solution is thoroughly mixed. This will not affect results, however if preferred the solution can be filtered using Whatman no.2 paper.*
4. *Add 4 Urea Peroxide tablets to 10 ml of the substrate solution. (alternatively add 40 µl 30% hydrogen peroxide.) This solution should be used within 2 hours.*

**3. OPD EASYtablets for ELISA**

OPD EASY (ortho-phylenediamine) tablets for ELISA contain also the citrate buffer giving you the proper pH(5.2) for immunoblotting when dissolved in water.

Short term storage at 4°C, long term storage at -20°C. Keep the lid tightly closed during storage of the tablets and do not remove the drying capsule from the container.

**How to use:**

1. *Remove the container from the refrigerator and allow to stand at room temperature for 15 minutes. DO NOT USE METAL FORCEPS DURING HANDLING OF THE TABLETS!*
2. *Dissolve 5 tablets, containing 2 mg OPD, in 12.5 ml of distilled water at room temperature.*
3. *Add 2 Urea Peroxide tablets to 10 ml of the substrate solution. (alternatively add 12.5 µl 30% hydrogen peroxide.) This solution should be used within 1/2 hour.*

**4. AEC EASYtablets**

AEC EASY (3-amino-9-ethyl carbazole) tablets for immunohistochemistry contain also the citrate buffer giving you the proper pH(5.1) for immunohistochemistry when dissolved in water.

Short term storage at 4°C, long term storage at -20°C. Keep the lid tightly closed during storage of the tablets and do not remove the drying capsule from the container.

#### How to use:

1. Remove the container from the refrigerator and allow to stand at room temperature for 15 minutes.  
*DO NOT USE METAL FORCEPS DURING HANDLING OF THE TABLETS!*
2. Add 1 ml of ethanol 96% to 1 tablet. Allow to rest for 3 minutes, add distilled water to a total volume of 10 ml at room temperature. The solution should be protected from direct sunlight.
3. Add 1 Urea Peroxide tablets to 10 ml of the substrate solution. (alternatively add 10 µl 30% hydrogen peroxide.) This solution should be used within 1 hour.

### 5. UREA PEROXIDE Tablets

UREA PEROXIDE tablets are a simple and convenient method of adding peroxide. To ease the use tablets are fizzing and dissolve in 5 minutes.

Each tablet contains 5.68 mg of urea-peroxide. When dissolved in 10 ml substrate solution of substrate of 6.0 µmol Urea-peroxide/ml.

**DO NOT USE METAL FORCEPS DURING HANDLING OF THE TABLETS!**

Short term storage at 4°C, long term storage at -20°C. Keep the lid tightly closed during storage of the tablets and do not remove the drying capsule from the container.

### 6. FAST RED Tablets

Fast red tablets usefull in immunohistochemistry an immunoblotting contain 0.5 mg naphthol substrate, 2 mg Fast Red chromogen and 0.4 mg levimasole.

Short term storage at 4°C, long term storage at -20°C. Keep the lid tightly closed during storage of the tablets and do not remove the drying capsule from the container.

#### How to use:

1. Remove tablets from refrigerator allowing 30 minutes to equilibrate to room temperature.
2. Prepare necessary substrate buffer (0.1M TRIS/HCL pH8.2). 12.1 g TRIS, add distilled water to 800 ml, adjust with 4 M HCL up to pH 8.2, add distilled water to final total volume of 1 Liter.
3. Use gloves and plastic forceps to handle tablets !
4. Add 1 tablet to 2 ml substrate buffer, shake for 3 minutes.
5. Some fine particular matter will remain in the suspension even after the solution is thoroughly mixed.  
*This will not affect results, however if preferred the solution can be filtered using Whatman no.2 paper.*
6. This solution should be used within 1/2 hour.

### 7. DAB tablets

DAB (3,3'-diaminobenzidine tetrahydrochloride) tablets are available in different sizes (2 and 10 mg/tablet).

Short term storage at 4°C, long term storage at -20°C. Keep the lid tightly closed during storage of the tablets and do not remove the drying capsule from the container.

### How to use:

1. Remove tablets from refrigerator allowing 15 minutes to equilibrate to room temperature.
2. DO NOT USE METAL FORCEPS DURING HANDLING OF THE TABLETS!
3. Prepare the necessary substrate buffer. Several buffer systems exist. DO NOT USE BUFFERS CONTAINING SODIUM AZIDE which is an inhibitor of HRP.
4. Dissolve 1 tablet in 10 ml of buffer (1 mg DAB/ ml). Some fine particular matter will remain in the suspension even after this DAB solution is thoroughly mixed. This will not affect results.
5. Add 20 $\mu$ l 30% hydrogen peroxide to 2 ml DAB solution. This solution should be used within 2 hours.
6. The remaining DAB solution that does not contain hydrogen peroxide can be stored in the dark for up to 5 days at 4°C.

### 8. OPD tablets

OPD EASY (ortho-phenylenediamine) are available in different sizes (2 and 10 mg/tablet) and guarantee a background as low as A490nm <0.05 in a concentration of 0.8 mg/ml (recommended substrate buffer : 0.05M citric acid/sodiumphosphate buffer).

Short term storage at 4°C, long term storage at -20°C. Keep the lid tightly closed during storage of the tablets and do not remove the drying capsule from the container.

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