

SERVALYT™ Carrier Ampholytes



Ampholytes are low molecular weight molecules of zwitterionic character. They are derived synthetically and comprise a multitude of varying pI-values. In agarose and polyacrylamide gels containing ampholytes, a linear pH gradient will be built up when an electric field is applied - the ampholyte molecules »carry« a net charge and thus migrate in the electric field between the electrodes as long as they will reach the position of corresponding pI. They will stop moving then and form small plateaus (stationary stacks).

SERVALYT™ ampholytes are a mixture of synthetically derived species of average molecular weight distribution of 400 to 1000 dalton.

Benefits are:

- high resolution due to multimeric composition
- fast staining and destaining times
- clear background associated with very low unspecific binding of dyes and stains
- high solubility in trichloroacetic acid (fast removal of ampholytes during fixation)
- virtually no interaction with metal ions

To achieve good separation of protein bands by IEF, stable pH-gradients with extensive and consistent buffer capacity are required. Good ampholyte mixtures comprise low molecular weight species of different pI-values which yield good conductivity at the isoelectric point. If the composition of the ampholyte buffer is maintained, reliable results and excellent reproducibility are assured for each experiment.

To achieve optimal resolution, the pI-range may be varied via the composition of the carrier ampholyte mixture in order to adjust the slope of the pH-gradient along the separation distance. Moreover, admixing narrow pH-cuts broadens the resulting pH-gradient which may improve the separation. This is particularly useful when complex samples are to be resolved featuring a »pI-focus«.

Wide range and narrow range SERVALYT™ ampholytes

The parameters listed above contribute to excellent separation if high resolution isoelectric focusing is to be achieved. SERVALYT™ carrier ampholytes are produced according to the highest quality standards and are routinely tested for performance. They are available as:

- wide range pH: spanning more than 3 pH units (e.g. SERVALYT™ 3-10)
- narrow range pH: fractions spanning 2 and 3 pH units (e.g. SERVALYT™ 3-6)
- ultra-narrow range pH: fractions spanning 1 pH units (e.g. SERVALYT™ 3-4)

Routine quality control constantly monitors the performance of SERVALYT™. Many criteria contribute to excellence but first of all the linearity of the gradient formed by the ampholyte throughout a gel and furthermore, the consistent staining/destaining characteristics after IEF is completed. Overall performance is judged by the final pherogramm

SERVALYT™ carrier ampholytes are supplied as 40 % aqueous concentrates, sterile filtered (0.2 µm). A common working concentration is in the range of 3 to 5 %. If stored at 4 °C, an unopened bottle of a ny SERVALYT™ stock is stable for up to 3 years. Technical grade SERVALYT™ carrier ampholyte pH 4-9 T (cat.no. 42910) is economical to use if preparative work is envisaged.

The »T« grade SERVALYT™ is not sterile filtered, UV absorption at 280 nm is higher than with analytical grade SERVALYT™ carrier ampholytes and buffer capacity at fringes is reduced. SERVALYT™ 3-10 IsoDalt (cat.no. 42951) is particularly suited to 2D-electrophoresis (Anderson et al. (1978) Anal. Biochem. 85, 33140; O'Farrell (1975) J. Biol. Chem. 250, 4007-21).

SERVALYT™ Carrier Ampholytes (40 % w/v solution in water)

All SERVALYT™ Types are of standard quality (analytical grade) for general use in I.E.F.

Bezeichnung	Kat.-Nr.	Quantity	Bezeichnung	Kat.-Nr..	Quantity
SERVALYT™ 2-4	42902.01	10 ml	SERVALYT™ 4-9 T 2)	42910.01	10 ml
	42902.02	25 ml		42910.02	25 ml
				42910.03	100 ml
SERVALYT™ 2-9 Seed-Mix	42935.01	10 ml	SERVALYT™ 5-6	42924.01	10 ml
	42935.02	25 ml		42924.02	25 ml
	42935.03	100 ml			
SERVALYT™ 2-11	42900.01	10 ml	SERVALYT™ 5-7	42905.01	10 ml
	42900.02	25 ml		42905.02	25 ml
SERVALYT™ 3-4	42922.01	10 ml	SERVALYT™ 5-7 PGM	42936.01	10 ml
	42922.02	25 ml		42936.02	25 ml
SERVALYT™ 3-5	42903.01	10 ml	SERVALYT™ 5-8	42949.01	10 ml
	42903.02	25 ml		42949.02	25 ml
SERVALYT™ 3-6	42944.01	10 ml	SERVALYT™ 5-9	42950.01	10 ml
	42944.02	25 ml		42950.02	25 ml
SERVALYT™ 3-7	42945.01	10 ml	SERVALYT™ 6-7	42925.01	10 ml
	42945.02	25 ml		42925.02	25 ml
SERVALYT™ 3-10 Standard	42940.01	10 ml	SERVALYT™ 6-8	42906.01	10 ml
	42940.02	25 ml		42906.02	25 m
SERVALYT™ 3-10 Iso-Dalt, for 2D ¹⁾	42951.01	10 ml	SERVALYT™ 6-9	42913.01	10 ml
	42951.02	25 ml		42913.02	25 ml
SERVALYT™ 4-5	42923.01	10 ml	SERVALYT™ 7-9	42907.01	10 ml
	42923.02	25 ml		42907.02	25 ml
SERVALYT™ 4-6	42904.01	10 ml	SERVALYT™ 9-11	42909.01	10 ml
	42904.02	25 ml		42909.02	25 ml
SERVALYT™ 4-7	42948.01	10 ml			
	42948.02	25 ml			

1) Iso-Dalt quality; special 2D-grade to be used in 2-D-lectrophoresis
2) Technical grade quality for preparative work

Note:

With some pH fractions, formation of solid particles can be found over time of storage at low temperature. This will not affect the separation as precipitate will dissolve upon dilution (working solution) or warming up to room temperature. Very acidic and alkaline fractions will slow down polymerization.

As a standard rule we recommend:

- in the case of acidic SERVALYT™ increase TEMED (cat. no. 35925) and APS (cat. no. 13375) to 0.1 % at a max. ampholyte concentration of 3 - 5 %
- in the case of alkaline fractions (above pH 9), reduce TEMED 0.05 % and increase APS to 0.1 % at a max. ampholyte concentration of 3 %.

Ready-to-use or preblending?

Wide range and narrow SERVALYT™ fractions are ready-to use. Generally, blending with other pH-fractions is not required but some exceptions apply which are important to know. Very acidic (pH 2-4) and very basic ampholytes (pH 7-9/9-11) may cause problems in polymerization. To circumvent this obstacle, we advise to add 20 % SERVALYT™ 3-10 (cat.no. 42940). Prior to casting, simply mix 8 ml of acidic or basic SERVALYT™ with 2 ml of wide range SERVALYT™. Moreover, the admixture assists at the edges of the gradient where strong pH-differences are built up between the electrode solutions in use and the ampholyte gradient.

With ultra-narrow pH-fractions (less than 1 pH unit) preblending may be recommendable. Depending on the choice of anode and cathode buffers sharp differences in pH may occur close to the edges of a gel (they could cause burning out and shunts). We recommend to add 20 % of the wide range SERVALYT™ 3-10 which will provide a smoother transition at the cathodic and anodic ends.

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