

MONOCLONAL ANTIBODY Estrogen Receptor

Cat. No.	Form	Quantity	Presentation
1344	Pre-diluted	6 mL	Ready-to-use
1545	Concentrated	1 mL	Liquid
2133	Concentrated	0.5 mL	Liquid

Clone ER1D5

Isotype IgG1 (mouse)

Immunogen Recombinant estrogen receptor (ER) protein of 67 kD.
The epitope is located on the N-terminal domain of ER (1).

Reagent preparation Pre-diluted antibody solution is ready-to-use use and should be employed according to experimental conditions and procedures validated by each individual laboratory.

Concentrated antibody solution should be diluted according to experimental conditions and procedures validated by each individual laboratory.

Recommended dilution: 1:50 for optimum quality.

Purity Pre-diluted form:
Purified Ig in 50 mM Tris-HCl, 150 mM NaCl, pH 7.2 with 1 mg/mL bovine serum albumin and 0.1 % sodium azide. The buffer contains a pink dye.

Concentrated forms:
Purified Ig in phosphate buffered saline with 2 mg/mL bovine serum albumin and 0.1 % sodium azide.

Storage 2-8°C until the expiration date stated on the vial label.

Warning Liquid reagents contain 0.1 % sodium azide. Under acidic conditions, sodium azide yields hydrazoic acid, an extremely toxic compound. Azide compounds should be flushed with copius amounts of water while being discarded in the drain. These precautions are recommended to avoid deposits in metal piping in which explosive conditions can develop.

Biological specimens Cytospins, smears and sections of fixed, paraffin-embedded tissues. Fixed, paraffin-embedded tissue sections require heating treatment prior to incubation with the antibody.

Tissue section heating procedure

Heating solution

10 mM citrate buffer, pH6 (2)
stock solution A: 0.1 M citric acid
stock solution B: 0.1 sodium citrate
Store at 2-8°C

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working solution: 9 mL of A + 41 mL of B, make up to 500 mL with deionized water

or

Citrate buffer powder, ready to dissolve in 1000 mL of deionized water, is provided under Cat. No. 1975.

Procedure*

Option 1: bring 3000 mL of citrate buffer heating solution to a boil in a pressure cooker, without the lid. Place the slides into the cooker, cover and lock the lid. Allow to boil under pressure for 4 minutes. Cool the unopened pressure cooker under tap water. Remove the slides and rinse in TBS or PBS (3).

This method is preferred since the heat treatment is independent of the number of slides treated. In addition, slides will not dry during the heating process.

Option 2: deparaffinized slides should be placed in a thermoresistant dish filled with citrate buffer. Run 3-5 cycles of 5 minutes each at 750 watts. Boiling is normally observed. Refill the dish with distilled water to replace evaporated water: sections should not dry. Remove the dish from the oven and allow to cool for 20 minutes at room temperature. Rinse slides in TBS or PBS buffer (2).

Note: to avoid bubble trapping between slides, it is recommended to leave at least 4 mm between slides.

* Depending on the exact protocol followed, this step may require a license under US. patent 5, 244, 787.

References

- 1) Al Saati, T., Clamens, S., Cohen-Knafo, E., Faye, J.C., Prats, H., Coindre, J.M., Wafflard, J., Caverivière, P., Bayard, F., Delsol, G., "Production of monoclonal antibodies to human recombinant estrogen-receptor protein (ER) using recombinant ER (RER)", 1993, Int. J. Cancer, **55**, 651-654.
- 2) Shi, S.R., Key, M.E., Kalra, K.L., "Antigen retrieval in formalin-fixed, paraffin-embedded tissues: an enhancement method for immunohistochemical staining based on microwave oven heating of tissue sections", 1991, J. Histochem. Cytochem., **39**, 6, 741-748.
- 3) Miller, K., Auld, J., Jessup, E., Rhodes, A., Ashton-Key, M., "Antigen unmasking in formalin-fixed routinely processed paraffin wax-embedded sections by pressure cooking: a comparison with a microwave oven heating and traditional methods", 1995, Advances in Anatomic Pathology, Ed., Raven Press, **2**, 1, 60-64.