H3R8Cit ELISA Capture Kit

Catalog No. R&D143001
Lot No. 20120001-28
Pack Size 96 reactions

Product Description:

Peptidyl arginine deiminase 4 (PAD4)-mediated citrullination of histone tail residues is a known marker of neutrophil extracellular trap (NET) formation and "NETosis" mediated cell death. NETs consist of citrullinated chromatin and associated granular proteins that are extruded from cells in the context of inflammation and disease. This kit includes a monoclonal antibody to histone H3 citrullinated at arginine 8 (H3R8Cit), validated against a panel of recombinant nucleosomes representing the physiological conformation of H3Cit in NETs (**Figure 1**). The antibody is provided in "ELISA Capture Kit" format with non-biotinylated H3R2,8,17Cit designer nucleosome (dNuc) for standard curve generation (**Figure 2**).

Kit Contents:

H3R8Cit Antibody, EpiCypher Catalog No. 13-0046, 25 µg
Recombinant rabbit monoclonal, Protein A affinity-purified antibody (0.96 mg/mL) in PBS, 40% glycerol. Not sold as a standalone product.

H3R2,8,17Cit dNuc, EpiCypher Catalog No. 16-1362k, 10 µL
Non-biotinylated recombinant human mononucleosomes citrullinated at H3R2, 8, and 17 (300 µg/mL). For additional information and representative quality control data, see EpiCypher Catalog No. 16-1362 at https://www.epicypher.com/.

Storage and Stability:

Stable for 1 year at -20°C from date of receipt. *NOTE: Avoid dNuc freeze/thaw. Upon first use, prepare 5 x 2 µL aliquots. Store at -20°C. Each aliquot is sufficient to prepare ELISA standard curve in duplicate.

Instructions for Use:

Sandwich ELISA: Dilute antibody 1:500 in 1X PBS. Add 100 µL/well to 96-well plate (*e.g.* Thermo Fisher Scientific High Bind Plates, Catalog No. 3855). Seal and incubate overnight at 4°C. Wash 3X with 50 mM Tris pH 7.5, 0.01% BSA, 0.01% Tween-20. Proceed to ELISA using the included H3R2,8,17Cit dNuc as a calibration standard (**Figure 2**) and the desired detection reagents (*e.g.* Thalin *et al.*, JTH 2020).

References:

Thalin *et al.*, (2020) JTH, doi:10.1111/jth.15003 ***NOTE: H3R8Cit antibody is cited as "R&D 13-0040, Lot 1" in the online pre-print***

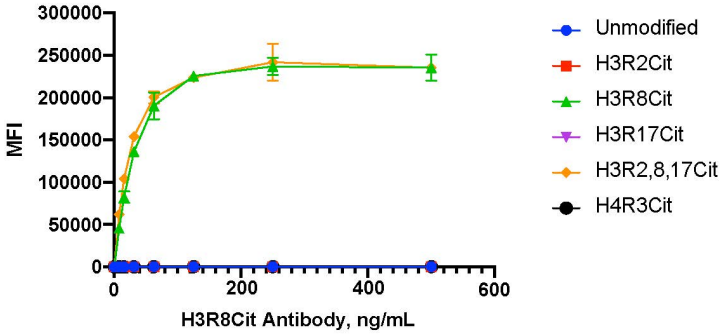

Figure 1: Antibody Specificity. H3R8Cit antibody was assessed using a Luminex® multiplexed approach employing a panel of recombinant nucleosomes with defined histone citrullinations at the indicated sites (legend, right). Each biotinylated nucleosome was individually coupled to Luminex MagPlex® beads. Antibody binding was tested in multiplex at the indicated concentrations (X-axis), and detected with second layer anti-IgG*PE. Luminex signal (MFI, FlexMAP 3D®) is shown (y-axis). H3R8Cit antibody specifically bound nucleosomes containing H3R8Cit when present alone and in combination with nearby citrulline modifications (H3R2,8,17Cit).

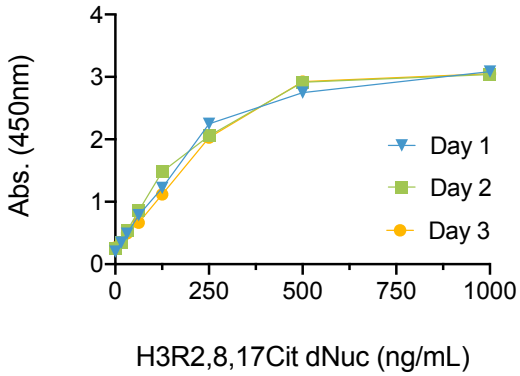

Figure 2: H3R8Cit ELISA dNuc Standard Curve. H3R8Cit antibody was coated onto 96-well plates at 1:500 dilution and used in sandwich ELISA. H3R2,8,17Cit dNuc was added at the indicated concentrations (x-axis) followed by addition of biotinylated H3R8Cit antibody (1:300 dilution). H3R8Cit signal (y-axis) was quantified using Streptavidin-HRP with 1-Step™ Ultra TMB-ELISA Substrate Solution (Thermo Fisher Scientific Catalog Nos. 21130 and 34028, respectively) using manufacturer's recommendations. Three representative calibration curves are shown (intra/inter-assay CVs are 4.4% and 11.4%, respectively).