

Quantifying Myeloperoxidase-DNA and Neutrophil Elastase-DNA Complexes from Neutrophil Extracellular Traps by Using a Modified Sandwich ELISA

Md. Monirul Islam¹, Umme Salma², Takayuki Irahara², Eizo Watanabe², Naoshi Takeyama²

¹Department of Biochemistry and Biotechnology, University of Science and Technology Chittagong (USTC) ²Department of Emergency and Critical Care Medicine, Aichi Medical University

Corresponding Author

Naoshi Takeyama

takeyama@aichi-med-u.ac.jp

Citation

Islam, M.M., Salma, U., Irahara, T., Watanabe, E., Takeyama, N. Quantifying Myeloperoxidase-DNA and Neutrophil Elastase-DNA Complexes from Neutrophil Extracellular Traps by Using a Modified Sandwich ELISA. *J. Vis. Exp.* (195), e64644, doi:10.3791/64644 (2023).

Date Published

May 12, 2023

DOI

10.3791/64644

URL

jove.com/video/64644

Materials

| Name | Company | Catalog Number | Comments |
|---|--|----------------|---|
| 1-Step Polymorphs | Accurate Chemical and Scientific Corporation | AN221725 | Isolation of PMN's from human blood. |
| 96-well microtiter plate | Thermo Fisher Scientific | 467466 | flat bottom |
| ABTS buffer solution | Sigma-Aldrich Merck | 11 204 530 001 | Contains sodium perborate, citric acid, and disodium hydrogen phosphate. |
| ABTS tablets | Sigma-Aldrich Merck | 11 204 521 001 | Each tablet contains 5 mg ABTS substrate and 60 mg vehicle substances. |
| Adhesive plastic cover, Axygen | Thermo Fisher Scientific | 14222348 | |
| Anti-MPO antibody | Sigma-Aldrich Merck | 07-496-I | Store at 2-8 °C. stable for 1 year. Host species is rabbit. |
| Anti-NE antibody, clone AHN-10 | Sigma-Aldrich Merck | MABS461 | Store at 2-8 °C. stable for 1 year. Host species is mouse. |
| Bovine serum albumin | Biomedical Science | BR-220700081 | Albumin from bovine fraction V. Store at 2-8 °C. stable for 2 year. |
| DNase I | New England BioLabs | M0303M | Store at -20 °C |
| IgG, rabbit, Isotype Control | GENETEX, Inc. | GTX35035 | Store as concentrated solution at 2-8 °C. |
| IgG1, mouse Isotype Control, clone Ci4 | Merck | MABC002 | Store as concentrated solution at 2-8 °C. |
| Lithium heparin blood collection tube | Becton Dickinson and Company | | |
| Microplate mixer | As one corporation | NS-P | |
| Microplate Reader | Molecular Devices | SpectraMax 190 | Any microplate plate reader capable of reading wavelengths from 405-490 nm can use. |
| Microplate reader application | Molecular Devices | SoftMax pro | |
| Peroxidase-conjugated anti-DNA antibody, Cell death Detection ELISA | Roche Diagnostics | 1154467500 | bottle 2. Store at 2-8 °C. stable for 1 year. |

| | | | |
|---|--|-----------|---|
| Phorbol 12-myristate 13-acetate | Sigma-Aldrich Merck | P8139 | Activation of PMN's from human blood. |
| Phosphate buffered solution | Takara Bio | T9181 | Store at room temperature. Stable for 6 months. |
| SigmaPlot v14.5 | Systat Software Inc. San Jose, CA, USA | | |
| Sodium azide | Fujifilm Wako Chemicals | 190-14901 | Store at room temperature. |
| t-Octylphenoxyethoxyethanol, Polyethylene glycol tert-octylphenyl ether | Fujifilm Wako Chemicals | 9002-93-1 | Store at room temperature. |