

VALIDATION DATA



NET Fragment Assay Kit (MPO-DNA)

Item No. 501330

The NET Fragment Assay Kit (MPO-DNA) was validated to assess its performance for the qualitative detection of NET fragments in biological matrices commonly used in research and preclinical studies. The validation experiments were designed to evaluate precision, accuracy, and matrix compatibility under the conditions described in the kit booklet.

The data presented in this document describe the performance characteristics of the assay and are intended to assist users in experimental planning and interpretation of results.

Parameter	Performance Metric
Detection method	Colorimetric (450 nm)
Assay duration	180 minutes
Sample volume	100 µl
Compatibility	Human
Validated species	Human
Validated matrices	Plasma (EDTA and citrate) and cell culture supernatants

WARNING

THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

SAFETY DATA

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the [complete](#) Safety Data Sheet, which has been sent via email to your institution.

WARRANTY AND LIMITATION OF REMEDY

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CAYMAN CHEMICAL

1180 EAST ELLSWORTH RD
ANN ARBOR, MI 48108 · USA

PHONE: [800] 364-9897
[734] 971-3335

FAX: [734] 971-3640

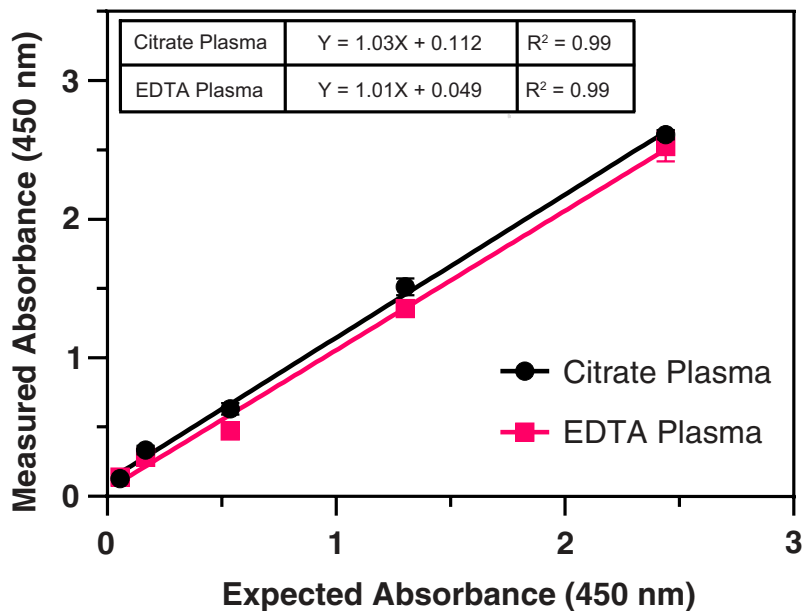
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WWW.CAYMANCHEM.COM

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Measured vs. Expected Absorbance in Assay

To evaluate assay performance in complex biological matrices, Assay Buffer or plasma samples were spiked with supernatant from polymorphonuclear leukocytes (PMNs) stimulated with phorbol 12-myristate 13-acetate (PMA) and A23187. Samples were diluted in Assay Buffer and analyzed using the assay.

Expected absorbance values were determined from samples prepared in Assay Buffer alone, while measured absorbance values were obtained from plasma-spiked samples. Measured absorbance values within 80–120% of the expected absorbance were considered acceptable. Values outside this range may indicate interference from matrix components.



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Dilutional Linearity

Linearity studies demonstrated that the absorbance intensity is directly proportional to the amount of NET fragments present in the sample. Serial dilutions of the samples demonstrated linear response and accurate qualification in validated matrices.

Human plasma spiked with PMA- and A23187-stimulated PMN supernatant and an A23187-stimulated PMN supernatant were analyzed at multiple dilutions using the NET Fragment Assay Kit (MPO-DNA).

Dilution Factor	Average Absorbance (450 nm)	% Linearity (Adjusted for dilution)
Spiked Citrate Plasma		
20	2.61	100
40	1.35	103
80	0.640	98.1
Spiked EDTA Plasma		
20	2.53	100
40	1.29	102
80	0.577	91.4
A23187-Stimulated PMN Supernatant		
100	2.97	100
200	1.37	92.3
400	0.730	98.3
800	0.345	92.9
1,600	0.177	95.4

Linearity in human plasma and cell culture samples

NOTE: Linearity has been calculated using the following formula: % Linearity = (Observed absorbance value * dilution factor correction/First observed absorbance concentration value in the dilution series) × 100

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Precision

The coefficient of variation (CV) is a measurement of consistency between sample replicates. Precision was evaluated both within a single assay run (intra-assay) and across multiple runs (inter-assay) on different days, using stimulated PMN cell culture supernatant (Control 1), unstimulated PMN cell culture supernatant (Control 2), and NET Fragment Control at a representative absorbance within the assay range.

Intra-Assay Precision: To assess intra-assay precision, a series of 24 replicate measurements were performed on the same day. The coefficients of variation (CVs) are listed in the table below.

	Absorbance (450 nm)	%CV
Control 1 (Stimulated PMN sample)	2.07	4.7
Control 2 (Unstimulated PMN sample)	0.500	6.1
NET Fragment Control	2.01	4.8

Inter-Assay Precision: To assess inter-assay precision, a series of eight measurements were performed on different days under the same experimental conditions. The resulting CVs are listed in the table below.

	Absorbance (450 nm)	%CV
Control 1 (Stimulated PMN sample)	1.46	13.5
Control 2 (Unstimulated PMN sample)	0.335	14.4
NET Fragment Control	2.04	6.5

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