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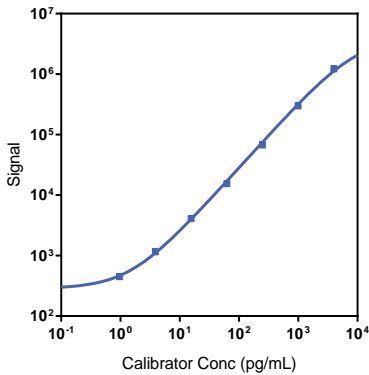
Company Address

MESO SCALE DISCOVERY®  
A division of  
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Product Options	Catalog Number	Description
Multiplex	K15067M, K25067M	U-PLEX Biomarker Group 1 (hu)
	K151AEM, K251AEM	U-PLEX Immuno-Oncology Group 1 (hu)
	K151ACM, K251ACM	U-PLEX Metabolic Group 1 (hu)
Singleplex	K151VEK-1/-2/-4	U-PLEX Human ENA-78 Assay with SECTOR™ plates
	K151VEK-21/-22/-24	U-PLEX Human ENA-78 Assay with QuickPlex® plates
	K151VEK-2/-4	U-PLEX Human ENA-78 Assay with 384-well plates
Antibody Set	B21VE-2/-3	U-PLEX Human ENA-78 Antibody Set
Protocol	U-PLEX Product Inserts are available at <a href="http://www.mesoscale.com">http://www.mesoscale.com</a>	

The U-PLEX® platform was designed to provide ultimate flexibility for detection of biomarkers in a wide variety of sample types. This datasheet provides the representative performance of the U-PLEX Human ENA-78 Assay tested on U-PLEX 96-well plates run as a multiplex. The data do not represent the product specifications. Under your experimental conditions, the assay may perform differently from the representative data. U-PLEX assays are offered in either singleplex or multiplex; both are available on 96- or 384-well plates. See a U-PLEX product insert for instrument compatibility.

Representative Calibration Curve and Sensitivity



Assay	Median LLOD (pg/mL)	LLOD Range (pg/mL)
ENA-78	0.53	0.31-1.1

The Calibrator curve was fitted with a 4-parameter logistic model with a 1/Y<sup>2</sup> weighting. The lower limit of detection (LLOD) is a calculated concentration corresponding to 2.5X the standard deviations above the background (zero Calibrator).

Precision

Control	Average Conc. (pg/mL)	Average Intra-run Conc. (%CV)	Inter-run Conc. (%CV)
High	1,730	3.9	6.2
Mid	144	3.3	7.8
Low	15	4.8	11.6

Controls were made by spiking Calibrator into assay diluent at 3 levels within the quantitative range of the assay. Average intra-run concentration %CV is the average %CV of the control replicates within an individual run. Inter-run concentration %CV is the variability of controls across multiple runs.

For Research Use Only.  
Not for use in diagnostic  
procedures.

# MSD® U-PLEX Human ENA-78

## Tested Samples

Sample Type	Serum (N=10)	Plasma (N=10)	Spiked Plasma (N=5)	Spiked Serum (N=5)
Median (pg/mL)	150	191	197	402
Range (pg/mL)	53–421	117–553	164–994	299–716
% Detected	100	100	100	100

Normal serum and plasma samples were tested without dilution prior to the assay.

## Dilution Linearity

Serum			EDTA Plasma		
Fold Dilution	Average % Recovery	% Recovery Range	Fold Dilution	Average % Recovery	% Recovery Range
2	103	97–108	2	99	93–104
4	109	102–119	4	98	92–105
8	111	104–123	8	93	83–104

Normal human serum and EDTA plasma were spiked with Calibrator and tested at different dilutions. Undiluted samples were tested to determine the expected concentration of the analyte. Samples may benefit from additional dilution with assay diluent to reduce matrix effects.

$$\% \text{ Recovery} = (\text{measured concentration} / \text{expected concentration}) \times 100$$

## Spike Recovery

Spike Level	Serum		EDTA Plasma	
	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range
High	89	86–91	101	98–103
Mid	93	87–96	99	87–104
Low	96	91–100	99	91–105

Normal serum and plasma were spiked with Calibrator at 3 levels. Undiluted samples were tested to determine the expected concentration of the analyte. Samples may benefit from additional dilution with assay diluent to reduce matrix effects.

$$\% \text{ Recovery} = (\text{measured concentration} / \text{expected concentration}) \times 100$$

## Specificity

To assess specificity, the ENA-78 Antibody Set was tested individually against a larger panel of analytes for nonspecific binding (APRIL/TNFSF13, BAFF, BAFF-R/TNFRSF13C, BCMA/TNFRSF17, BDNF, C-Peptide, CD20, CD27, CD28, CD40L (soluble), CD276/B7-H3, CTACK, CTLA-4, Desghrelin, ENA-78, Eotaxin, Eotaxin-2, Eotaxin-3, EPO, E-Selectin, FGF (basic), FGF-21, FGF-23, FLT3L, Fractalkine, FSH, Galectin-9, G-CSF, GITRL/TNFSF18, GPCR/TNFRSF18, Ghrelin (Ser3-octanoylated), gp130 (soluble), GIP (1–42), GIP (3–42), GLP-1 (7–36), GLP-1 (9–36), Glucagon, GM-CSF, Granzyme A, Granzyme B, GRO- $\alpha$ , HAVCR2/TIM-3, HVEM/TNFRSF14, ICOS, ICOS-L/B7-H2, I-309, IFN- $\alpha$ 2a, IFN- $\beta$ , IFN- $\gamma$ , IL-1 $\alpha$ , IL-1 $\beta$ , IL-1RA, IL-2, IL-2R $\alpha$ , IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12/IL-23p40, IL-12p70, IL-13, IL-15, IL-16, IL-17A, IL-17A/F, IL-17C, IL-17D, IL-17E/IL-25, IL-17F, IL-18, IL-21, IL-22, IL-23, IL-27, IL-29/IFN- $\lambda$ 1, IL-31, IL-33, Insulin, IP-10, LAG3, Leptin, LH, LIGHT/TNFSF14, MCP-1, MCP-2, MCP-4, M-CSF, MDC, MIF, MIG, MIP-1 $\alpha$ , MIP-1 $\beta$ , MIP-5, MMP-1, MMP-2, MMP-7, MMP-9 (total), Nectin-4, OX40/TNFRSF4, PD1, PD-L1, PD-L2, Pentraxin 3, Perforin, PIGF, PP, Proinsulin, proMMP-9, P-Selectin, PYY (3–36), RAGE (soluble), RANKL/TNFSF11, RANTES, S100A12, SDF-1 $\alpha$ , Tie-2, TIGIT, TLR1, TNF- $\alpha$ , TNF- $\beta$ , TNF-RI, TNF-RII, TPO, TRAIL, TSLP, VEGF-A, VEGF-D, VEGFR-1/Flt-1, YKL-40, and  $\beta$ -NGF). Nonspecific binding was less than 2.0%.

$$\% \text{ Nonspecificity} = (\text{nonspecific signal} / \text{specific signal}) \times 100$$

## Diluent Compatibility

Diluents 57 and 3 are provided with this assay. MSD offers a range of assay and antibody diluents for separate purchase. Depending on your assay needs, other diluents may be tested.

## Assay Components

**Calibrator:** ENA-78 is included in Calibrator 4. The ENA-78 Calibrator is a full-length recombinant protein expressed in *E. coli*.

**Antibodies:** The U-PLEX Human ENA-78 Assay uses a mouse monoclonal antibody for capture and a goat polyclonal antibody for detection.

**Assay generation:** A

**Note:** This datasheet contains representative assay performance data. In custom multiplex formats, the assay may perform differently from the representative data shown.

