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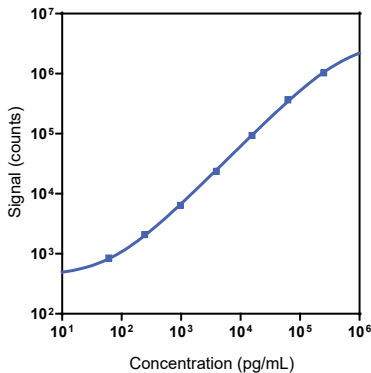
Company Address

MESO SCALE DISCOVERY®
A division of
Meso Scale Diagnostics, LLC.
1601 Research Boulevard
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Product Options	Catalog Number	Description
Multiplex	K151AEM, K251AEM	U-PLEX Immuno-Oncology Group 1 (human)
Singleplex	K151H7K-1/-2/-4	U-PLEX Human CD28 Assay with SECTOR™ plates
	K151H7K-21/-22/-24	U-PLEX Human CD28 Assay with QuickPlex Ultra™ plates
	K251H7K-2/-4	U-PLEX Human CD28 Assay with 384-well plates
Antibody Set	B21H7-2/-3	U-PLEX Human CD28 Antibody Set
Protocol	U-PLEX Product Inserts are available at www.mesoscale.com	

The MESO SCALE DISCOVERY® 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 CD28 Assay tested on U-PLEX 96-well SECTOR 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)
CD28	14	5.1-26

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

Precision

Control	Average Conc. (pg/mL)	Average Intra-run Conc. (%CV)	Inter-run Conc. (%CV)
High	30,200	4.7	6.3
Mid	4,780	2.3	8.1
Low	736	4.3	14.6

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 CD28

Tested Samples

Sample Type	Serum (N=10)	EDTA Plasma (N=10)	Normal Lysate (N=5)	Tumor Lysate (N=5)
Median (pg/mL)	135	167	172	142
Range (pg/mL)	70-179	80-369	95-702	13-252
% Detected	100	100	100	100

Normal serum and plasma samples were diluted 4-fold prior to the assay. Lysates were tested at a protein concentration of 0.5 mg/mL.

Dilution Linearity

Serum			EDTA Plasma		
Fold Dilution	Average % Recovery	% Recovery Range	Fold Dilution	Average % Recovery	% Recovery Range
2	97	90 - 102	2	100	89 - 109
8	98	97 - 98	8	96	93 - 99
16	96	91 - 101	16	94	91 - 99

Normal human serum and EDTA plasma were spiked with Calibrator and tested at different dilutions. Percent recovery at each dilution level was normalized to the dilution-adjusted, 4-fold concentration. 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	80 - 99	84	68 - 94
Mid	82	42 - 105	89	68 - 101
Low	110	83 - 168	85	65 - 97

Normal serum and plasma were spiked with Calibrator at 3 levels. Spiked samples were diluted 4-fold 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 CD28 Antibody Set was tested individually against a larger panel of analytes for nonspecific binding: APRIL/TNFSF13, BAFF-R/TNFRSF13C, BCMA/TNFRSF17, CD20, CD27, CD276/B7-H3, CD28, CD40L (soluble), CTACK, CTLA-4, ENA-78, Eotaxin, Eotaxin-2, Eotaxin-3, EPO, E-Selectin, FGF (basic), FLT3L, Fractalkine, G-CSF, Galectin-9, GITR/TNFRSF18, GITRL/TNFSF18, GM-CSF, gp130 (soluble), Granzyme A, Granzyme B, GRO- α , HAVCR2/TIM-3, HVEM/TNFRSF14, I-309, ICOS, ICOSL/B7-H2, IFN- α 2a, IFN- β , IFN- γ , IL-1 α , IL-1 β , IL-1RA, 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-2, IL-21, IL-22, IL-23, IL-27, IL-29/IFN- λ 1, IL-2R α , IL-3, IL-31, IL-33, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IP-10, I-TAC, LAG-3, LIGHT/TNFSF14, MCP-1, MCP-2, MCP-4, M-CSF, MDC, MIF, MIG, MIP-1 α , MIP-5, MMP-1, MMP-2, MMP-7, MMP-9, Nectin-4, OX40/TNFRSF4, PD1, PD-L1, PD-L2, Pentraxin 3, Perforin, PIGF, P-Selectin, RAGE (soluble), RANKL/TNFSF11, RANTES, S100A12, SDF-1 α , TARC, Tie-2, TIGIT, TLR-1, TNF-RI, TNF-RII, TNF- α , TNF- β , TPO, TRAIL, TSLP, VEGF-A, VEGF-D, VEGFR-1/Flt-1 and YKL-40. Nonspecific binding was less than 2.0%.

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

Diluent Compatibility

Diluents 58 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: CD28 is included in Calibrator 20 The human CD28 Calibrator is CD28 (19–152) protein expressed in a mouse cell line.

Antibodies: The U-PLEX Human CD28 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.

