MSD® MULTI-SPOT Assay System

Human Tenascin C Kit

1-Plate Kit	K151OJD-1
5-Plate Kit	K151OJD-2
25-Plate Kit	K151OJD-4

MSD Biomarker Assays

Human Tenascin C Kit

This package insert must be read in its entirety before using this product.

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

MESO SCALE DISCOVERY®
A division of Meso Scale Diagnostics, LLC.
1601 Research Boulevard
Rockville, MD 20850-3173 USA
www.mesoscale.com

Table of Contents

Ordering Information

MSD Customer Service

Phone: 1-301-947-2085
Fax: 1-301-990-2776
Email: CustomerService@mesoscale.com

MSD Scientific Support

Phone: 1-301-947-2025
Fax: 1-240-632-2219 attn: Scientific Support
Email: ScientificSupport@mesoscale.com

Introduction

The tenascins are a group of extracellular matrix glycoproteins involved in vertebrate development, tissue injury, and repair. Tenascin C is the founding and best characterized member of this group of proteins originally found in gliomas, muscle tissue, and the nervous system. The variety of locations in which it was found gave rise to its alternate names: myotendinous antigen, glial/mesenchymal ECM protein, cytoactin, J1 220/200, neuronectin, and hexabrachion.[1] It was also later found in the osteotendinous junction and superficial layers of cartilage.[2,3]

Tenascin C is a homohexamer (1500 kDa) formed through disulfide linkages in its N-terminal domain.[4] Its structure is composed of an N-terminal oligomerization domain, multiple EGF-like repeats, fibronectin type III repeats, and a C-terminal fibrinogen-like globular domain. Through these domains and interactions with fibronectin, tenascin C interacts with a wide variety of other extracellular matrix molecules and receptors including integrins alpha-8/beta-1, alpha-9/beta-1, alpha-V/beta-3, and alpha-V/beta-6. Functionally, it regulates cell adhesion, migration, proliferation, and cellular signaling.[5]

Tenascin C is highly expressed during development for embryogenesis and organogenesis, then reduces to undetectable levels in adults; however, its expression increases in adults during wound healing and neoplastic events.[6,7] A number of pathologies are also associated with an upregulation of tenascin C expression including cardiac injury, and various tumors (glioblastoma, breast, colon, and oral cancer). In a subset of tumors, higher levels of tenascin C correlate with greater metastatic incidence and poorer prognosis.[8] Complicating the analysis of tenascin C in these diseases is the fact that it occurs in a wide variety of isoforms in a disease-specific manner.[9] These isoforms are based on alternative splicing in the fibronectin type III repeats producing size and functionally distinct tenascin C proteins. While the shortest isoform is present in adult cartilage, up to 27 different splicoforms are temporally and spatially regulated in the developing mouse. Due to the large differences in isoform specific expression during disease onset and progression, the measurement of tenascin C levels is of interest as a potential biomarker.

Principle of the Assay

MSD biomarker assays provide a rapid and convenient method for measuring the levels of protein targets within a single, small-volume sample. Human Tenascin C is a sandwich immunoassay (Figure 1). MSD provides a plate pre-coated with capture antibodies. The user adds the sample and a solution containing detection antibodies conjugated with electrochemiluminescent labels (MSD SULFO-TAG™) over the course of one or more incubation periods. Analytes in the sample bind to capture antibodies immobilized on the working electrode surface; recruitment of the detection antibodies by the bound analytes completes the sandwich. The user adds an MSD buffer that provides the appropriate chemical environment for electrochemiluminescence and loads the plate into a SECTOR® Imager where a voltage applied to the plate electrodes causes the captured labels to emit light. The instrument measures the intensity of emitted light to provide a quantitative measure of analytes in the sample.

1. Tenascin C
2. *BSA blocked*
3. *BSA blocked*
4. *BSA blocked*

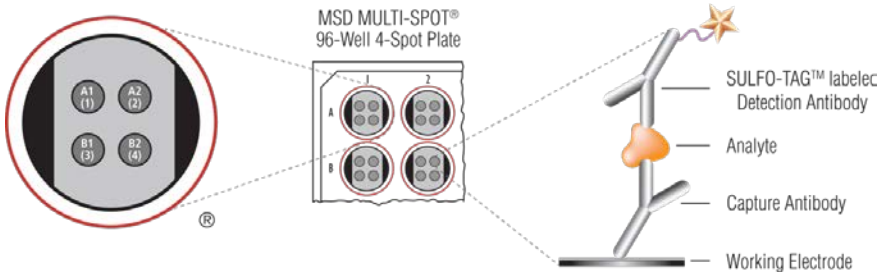


Figure 1. *Spot diagram showing placement of analyte capture antibodies. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files. A unique bar code label on each plate allows complete traceability back to MSD manufacturing records.*

Reagents Supplied

Product Description	Storage	Quantity per Kit K4510JD-1	K4510JD-2	K4510JD-4
MULTI-SPOT 96-Well, 4-Spot Human Tenascin C Plate N4510JA-1	2–8°C	1 plate	5 plates	25 plates
SULFO-TAG Anti-hu Tenascin C Antibody[1] (50X)	2–8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea)
Human Tenascin C Calibrator (20X)	≤-70°C	1 vial (60 µL)	5 vials (60 µL ea)	25 vials (60 µL ea)
Diluent 100 R50AA-2 (200 mL)	2–8°C	1 bottle (200 mL)	2 bottles (200 mL ea)	10 bottles (200 mL ea)
Diluent 3 R51BA-4 (5 mL), R51BA-5 (25 mL)	≤-10°C	1 bottle (5 mL)	1 bottle (25 mL ea)	5 bottles (25 mL ea)
Blocker A Kit (Blocker A [dry] in 250 mL bottle and 50 mL bottle of 5X Phosphate Buffer) R93AA-2 (250 mL)	RT	1 kit (250 mL)	1 kit (250 mL)	5 kits (250 mL ea)
Read Buffer T (4X) R92TC-3 (50 mL)	RT	1 bottle (50 mL)	1 bottle (50 mL)	5 bottles (50 mL ea)

Required Material and Equipment (not supplied)

- ❑ Appropriately sized tubes for reagent preparation
- ❑ Microcentrifuge tubes for preparing serial dilutions
- ❑ Phosphate-buffered saline plus 0.05% Tween-20 (PBS-T) for plate washing
- ❑ Liquid handling equipment for desired throughput, capable of dispensing 10 to 150 µL/well into a 96-well microtiter plate
- ❑ Plate washing equipment: automated plate washer or multichannel pipette
- ❑ Adhesive plate seals
- ❑ Microtiter plate shaker
- ❑ Deionized water

Safety

Use safe laboratory practices and wear gloves, safety glasses, and lab coats when handling kit components. Handle and dispose of all hazardous samples properly in accordance with local, state, and federal guidelines.

Additional safety information is available in the product Material Safety Data Sheet, which can be obtained from MSD Customer Service.

[1] SULFO-TAG–conjugated detection antibodies should be stored in the dark.

Reagent Preparation

Bring all reagents to room temperature. Thaw the stock calibrator on ice.

> **Important:** Upon first thaw, separate Diluent 3 into aliquots appropriate for the size of your needs before refreezing.

Prepare Blocker A Solution

Follow the Blocker A instructions included in the kit.

Prepare Standards

MSD supplies calibrator for the Human Tenascin C Kit at 20-fold higher concentration than the recommended highest standard. We recommend a 7-point standard curve with 3-fold serial dilution steps and a zero calibrator blank. Signals from the blank should be excluded when generating the curve. Thaw the stock calibrator and keep on ice, then add to diluent at room temperature to make the standard curve solutions.

Standard	Human Tenascin C (pg/mL)	Dilution Factor
Stock Calibrator	2500	
STD-01	125	20
STD-02	41.7	3
STD-03	13.9	3
STD-04	4.63	3
STD-05	1.54	3
STD-06	0.514	3
STD-07	0.171	3
STD-08	0	n/a

To prepare 7 standard solutions plus a zero calibrator blank for up to 3 replicates:

1) Prepare the highest standard by adding 15 µL of stock calibrator to 285 µL of Diluent 100. Mix well.
2) Prepare the next standard by transferring 100 µL of the highest standard to 200 µL of Diluent 100. Mix well. Repeat 3-fold serial dilutions 5 additional times to generate 7 standards.
3) Use Diluent 100 as the blank.

Dilute Samples

For human serum and plasma samples, MSD recommends 4000-fold dilution in Diluent 100; however, you may need to adjust the dilution factor for the sample set under investigation.

Samples should be prepared in two dilution steps as follows:

1) Add 10 µL of sample to 390 µL of Diluent 100 (40-fold dilution)
2) Add 10 µL of the 40-fold diluted sample to 990 µL of Diluent 100 (100-fold dilution).

Prepare Detection Antibody Solution

MSD provides detection antibody as a 50X stock solution. The working detection antibody solution is 1X.

For 1 plate, combine:

- ❑ 60 µL of 50X SULFO-TAG Anti-hu Tenascin C Antibody
- ❑ 2940 µL of Diluent 3

Prepare Read Buffer

MSD provides Read Buffer T as a 4X stock solution. The working solution is 1X.

For 1 plate, combine:

- ❑ 5 mL of Read Buffer T (4X)
- ❑ 15 mL of deionized water

You may prepare diluted read buffer in advance and store it at room temperature in a tightly sealed container.

Prepare MSD Plate

MSD plates are pre-coated with capture antibodies (Figure 1) and exposed to a proprietary stabilizing treatment to ensure the integrity and stability of the immobilized antibodies. Plates can be used as delivered; no additional preparation (e.g., pre-wetting) is required.

Protocol

Notes

1. **Add Blocker A Solution:** Add 150 µL of Blocker A solution to each well. Seal the plate with an adhesive plate seal and incubate for 1 hour with vigorous shaking (300–1000 rpm) at room temperature.

Shaking the plate typically accelerates capture at the working electrode.

2. **Wash and Add Sample:** Wash the plate 3 times with 300 µL/well of PBS-T. Add 50 µL of sample (standards, controls, or unknowns) per well. Seal the plate with an adhesive plate seal and incubate for 2 hours with vigorous shaking (300–1000 rpm) at room temperature.
   You may prepare detection antibody solution during incubation.

3. **Wash and Add Detection Antibody Solution:** Wash the plate 3 times with 300 µL/well of PBS-T. Add 25 µL of detection antibody solution to each well. Seal the plate with an adhesive plate seal and incubate for 1 hour with vigorous shaking (300–1000 rpm) at room temperature.
   You may prepare diluted read buffer during incubation.

You may keep excess diluted read buffer in a tightly sealed container at room temperature for later use.

Bubbles introduced when adding read buffer will interfere with imaging of the plate and produce unreliable data. Use reverse pipetting technique to avoid creating bubbles.

4. **Wash and Read:** Wash the plate 3 times with 300 µL/well of PBS-T. Add 150 µL of 1X Read Buffer T to each well. Analyze the plate on the SECTOR Imager. No incubation in read buffer is required before reading the plate.

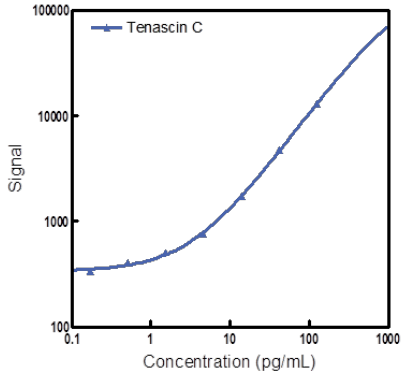
Due to the varying nature of each research application, you should assess assay stability before allowing plates to sit with read buffer for extended periods.

Curve Fitting

MSD DISCOVERY WORKBENCH® software uses least-squares fitting algorithms to generate the standard curve that will be used to calculate the concentration of analyte in the samples. The assays have a wide dynamic range (3–4 logs) that allows accurate quantification without the need for dilution in many cases. By default, the software uses a 4-parameter logistic model (or sigmoidal dose-response) and includes a $1/Y^2$ weighting function. The weighting function is important because it provides a better fit of data over a wide dynamic range, particularly at the low end of the standard curve.

Typical Data

The following standard curve graph illustrates the dynamic range of the assay. Actual signals will vary. Best quantification of unknown samples will be achieved by generating a standard curve for each plate using a minimum of 2 replicates of the standards.



Tenascin C		
Conc. (pg/mL)	Average Signal	%CV
0	339	5.2
0.171	337	0.8
0.514	404	4.9
1.54	506	3.6
4.63	758	0.1
13.9	1732	2.0
41.7	4680	9.3
125	12 875	1.5

Sensitivity

The lower limit of detection (LLOD) is a calculated concentration based on a signal 2.5 standard deviations above the background (zero calibrator blank). The LLOD shown below was calculated based on 2 runs.

	Tenascin C
LLOD Range (pg/mL)	0.442–0.477

Assay Components

Calibrator

The assay calibrator uses recombinant Tenascin C protein, residues 23–625,expressed in murine myeloma cells.

Antibodies

	Source Species	
Analyte	MSD Capture Antibody	MSD Detection Antibody
Tenascin C	Rat monoclonal	Goat polyclonal

References

1. Chiquet-Ehrismann R, Chiquet M. Tenascins: regulation and putative functions during pathological stress. J Pathol. 2003 Jul;200(4):488-99.
2. Mackie EJ, et al. Tenascin is associated with chondrogenic and osteogenic differentiation in vivo and promotes chondrogenesis in vitro. J Cell Biol. 1987 Dec;105(6 Pt 1):2569-79.
3. Mackie EJ, Tucker RP. Tenascin in bone morphogenesis: expression by osteoblasts and cell type-specific expression of splice variants. J Cell Sci. 1992 Nov;103 (Pt 3):765-71.
4. Midwood KS, et al. Advances in tenascin-C biology. Cell Mol Life Sci. 2011 Oct;68(19):3175-99.
5. Chiquet-Ehrismann R, Chiquet M. Tenascins: regulation and putative functions during pathological stress. J Pathol. 2003 Jul;200(4):488-99.
6. Ballard VL, et al. Vascular tenascin-C regulates cardiac endothelial phenotype and neovascularization. FASEB J. 2006 Apr;20(6):717-9..
7. Jones FS, Jones PL. The tenascin family of ECM glycoproteins: structure, function, and regulation during embryonic development and tissue remodeling. Dev Dyn. 2000 Jun;218(2):235-59.
8. Brellier F, Chiquet-Ehrismann R. How do tenascins influence the birth and life of a malignant cell? J Cell Mol Med. 2012 Jan;16(1):32-40.
9. Joester A, Faissner A. Evidence for combinatorial variability of tenascin-C isoforms and developmental regulation in the mouse central nervous system. J Biol Chem. 1999 Jun 11;274(24):17144-51.

Summary Protocol

Human Tenascin C Kit

MSD provides this summary protocol for your convenience. Please read the entire detailed protocol prior to performing the Human Tenascin C assay.

Sample and Reagent Preparation

Bring all reagents to room temperature and thaw the calibrator on ice.

Prepare Blocker A solution.

Prepare standard solutions using the supplied calibrator:

- Dilute the stock calibrator 20-fold in Diluent 100.
- Perform a series of 3-fold dilution steps and prepare a zero calibrator blank.

Dilute samples 4000-fold in Diluent 100 before adding to the plate.

Prepare detection antibody solution by diluting stock detection antibody 50-fold in Diluent 3.

Prepare 1X Read Buffer T by diluting stock 4X Read Buffer T 4-fold with deionized water.

Step 1: Add Blocker A Solution

Add 150 µL/well of Blocker A solution.

Incubate at room temperature with vigorous shaking (300–1000 rpm) for 1 hour.

Step 2: Wash and Add Sample

Wash plate 3 times with 300 µL/well of PBS-T.

Add 50 µL/well of sample (standards, controls, or unknowns).

Incubate at room temperature with vigorous shaking (300–1000 rpm) for 2 hours.

Step 3: Wash and Add Detection Antibody Solution

Wash plate 3 times with 300 µL/well of PBS-T.

Add 25 µL/well of 1X detection antibody solution.

Incubate at room temperature with vigorous shaking (300–1000 rpm) for 1 hour.

Step 4: Wash and Read Plate

Wash plate 3 times with 300 µL/well of PBS-T.

Add 150 µL/well of 1X Read Buffer T.

Analyze plate on SECTOR Imager.

Plate Diagrams