

## INNOVO® Assay Sharing

# Data Report (Demo): Hepatic Stellate Cell Fibrosis Assay

Report Date: 6<sup>th</sup> June 2021

Compound Number: 3

Assays: Renal Mesangial Cell Fibrosis Assay  
Hepatic Stellate Cell Fibrosis Assay  
Cardiac Fibroblast Fibrosis Assay

# Contents

1. Compound List	4
2. Experiment Protocol	5
Cell Culture (Day 0)	5
Treatment (Day 1)	5
Cell Lysis (Day 2/4)	5
Reverse Transcription	6
RT-qPCR	6
3. Explanation of the data report	7
4. Data Report	8
Compound 1: TestMol_1	8
Compound 2: TestMol_3	9
5. Conclusion	10

Thank you for participating in our INNOVO® Assay Sharing Program by submitting your compounds for evaluation in our disease-relevant *in vitro* assays.

We hereby represents that, to the best of its knowledge, the data and information in the report provided to you will be accurate and what it purports to be. However it's important to note that these assays are not representatives of all conceivable disease mechanisms. It is possible that you find the results are incongruous with your observations in other models. These results are prepared by us solely for the purpose of facilitating your research and of exploring the opportunity of future collaboration. You are the owner of these data and may use them for any purpose. If you plan to publish them, please acknowledge that "Assay test data provided by Novo Nordisk under the INNOVO® Assay Sharing Program". Should you have any questions or suggestions, please feel free to contact [assaysharing@novonordisk.com](mailto:assaysharing@novonordisk.com).

# 1. Compound List

Compounds Name	Compounds ID	QC Results	Molecular Weight (kDa)	Remarks	.....
TestMol_1	ASXX123_01	Pass	50		
TestMol_2	ASXX123_02	Fail	-	Not pure, insufficient total amount.	
TestMol_3	ASXX123_03	Pass	54		

## 2. Experiment Protocol

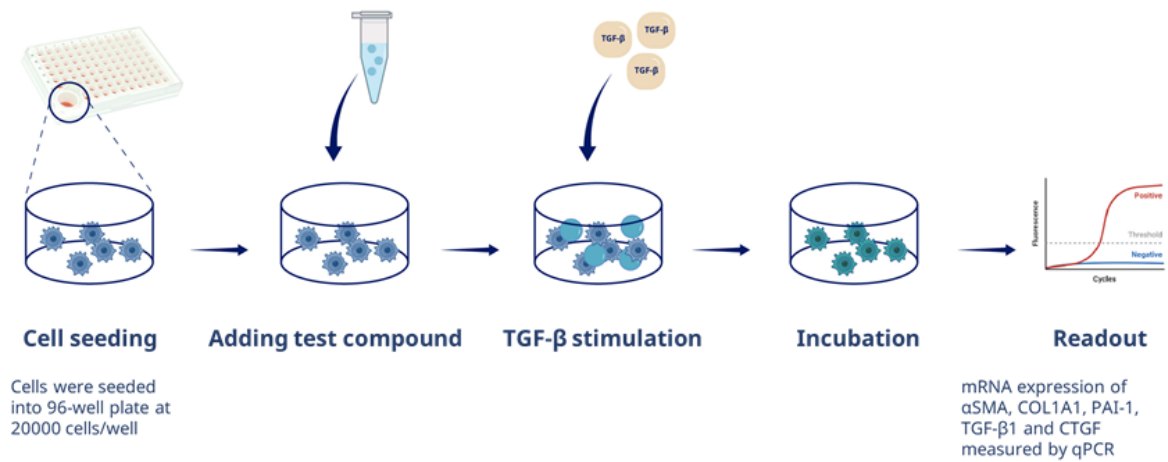


Figure 1. Experiment workflow.

### Cell Culture (Day 0)

- **Cell Seeding:**  
For 96 well plate: Seed LX-2 cells at density of 20000 cells/well in 96 well plates (Corning PDL-plate, cat# 3842) in 2% FBS complete medium, 200µL/well.  
Or, for 384 well plate: Seed LX-2 cells at density of 5000 cells/well in 384 well plates in 2% FBS complete medium, 50µL/well.
- Incubate 24 hours at 37°C and 5% CO<sub>2</sub>, avoid opening/closing the incubator.

### Treatment (Day 1)

- For peptide/protein/small molecules:  
Cells will be treated with compound at indicated concentrations. 30 min later, add TGF-β in duplicate at a final concentration of 5ng/mL of assay medium, 200 µL/well (50 µL/well for 384 well plate). Incubate at 37°C with 5% CO<sub>2</sub>.
- For siRNA or ASO compounds:  
Cell will be transfected with 20 nmol siRNA/ASO. At day3, add TGF-β in duplicate at a final concentration of 5ng/ml of assay medium, 200 µL/well (50 µL/well for 384 well plate). Incubate at 37°C with 5% CO<sub>2</sub>.

### Cell Lysis (Day 2/4)

- After 24 h stimulation with TGF-β, wash cells with ice-cold DPBS (free of Mg<sup>2+</sup>/Ca<sup>2+</sup>).
- Prepare Lysis Solution:

Component	Volume (µL)	Or	Volume (µL)
<b>Lysis Solution</b>	49.5		14.85
<b>DNase I</b>	0.5		0.15
<b>In total</b>	50 per rxn		15 per rxn

- Add 50 µL (15 µL for 384 well plate) Lysis Solution to each well of cells, incubate for 5 min on a horizontal microplate shaker (900 rpm) at room temperature (19-25°C).
- Add 5 µL Stop Solution directly into each lysis reaction and continue incubating for 2 min on a horizontal microplate shaker (900 rpm).
- Cell lysates are applied in RT process subsequently (or can be stored at -80°C until use).

Reverse Transcription

- Prepare RT Master Mix:

Component	Volume (µL)
<b>2X SYBR® RT Buffer</b>	10
<b>20X RT Enzyme Mix</b>	1
<b>Nuclease-free water</b>	3
<b>In total</b>	14 per rxn

- Distribute 14 µL RT Master Mix to a RT plate. Add 6 µL sample lysate to each aliquot of RT Master Mix for a final 20 µL reaction volume and mix thoroughly, each reaction in duplicate.
- Run RT program on a PCR machine, RT products will be applied in a PCR process subsequently (or can be stored at -20°C until use):

Step	RT Process	Temp. (°C)	Time (min)	Repeat
<b>1</b>	Reverse Transcription	37	60	1
<b>2</b>	RT Inactivation	95	5	1
<b>3</b>	Hold	4	∞	1

RT-qPCR

- Prepare PCR Master Mix for target genes (αSMA, COL1A1, PAI-1, TGF-β1, CTGF) and endogenous control genes (GAPDH) respectively.
- For 384 well plate (10 µL system):

Component	Volume (ul)	Conc.
<b>2X SYBR Green Master Mix</b>	5	2X
<b>Primer Set Mix (2.5 µM)</b>	1	10X
<b>Nuclease-free Water</b>	2	N/A
<b>In total</b>	8 per rxn	N/A

- Distribute 8 µL PCR Master Mix to an assay plate and add 2 µL RT product samples to each aliquot of PCR Master Mix in duplicate.
- Spin the plate (1000 rpm, 2min) and run PCR program on Applied Biosystems ViiA7 instrument.

### 3. Explanation of the data report

Your results are reported with tables of raw data and values normalized to a housekeeping gene. Graphs are plotted based on the normalized values.

#### Raw data:

Raw data are the average Ct values from RT-qPCR experiments. For each sample, Ct values of GAPDH the house keeping gene and the fibrosis related gene panel are recorded.

#### Table of normalized values:

The values are used to generate the Graph. Each data point represents the relative mRNA expression level of individual gene compared to GAPDH followed by power calculation.

#### Graph:

The plot is automatically generated illustrating the normalized mRNA expression level relative to GAPDH and the fitted curve. Specifically, it depicts the mRNA level change of a panel fibrosis related genes.

# 4. Data Report

Compound 1: TestMol\_1  
 Compounds ID: ASXXX123\_01  
 Molecular Weight: 50 kDa  
 Assay Name: Hepatic Stellate Cell Fibrosis Assay

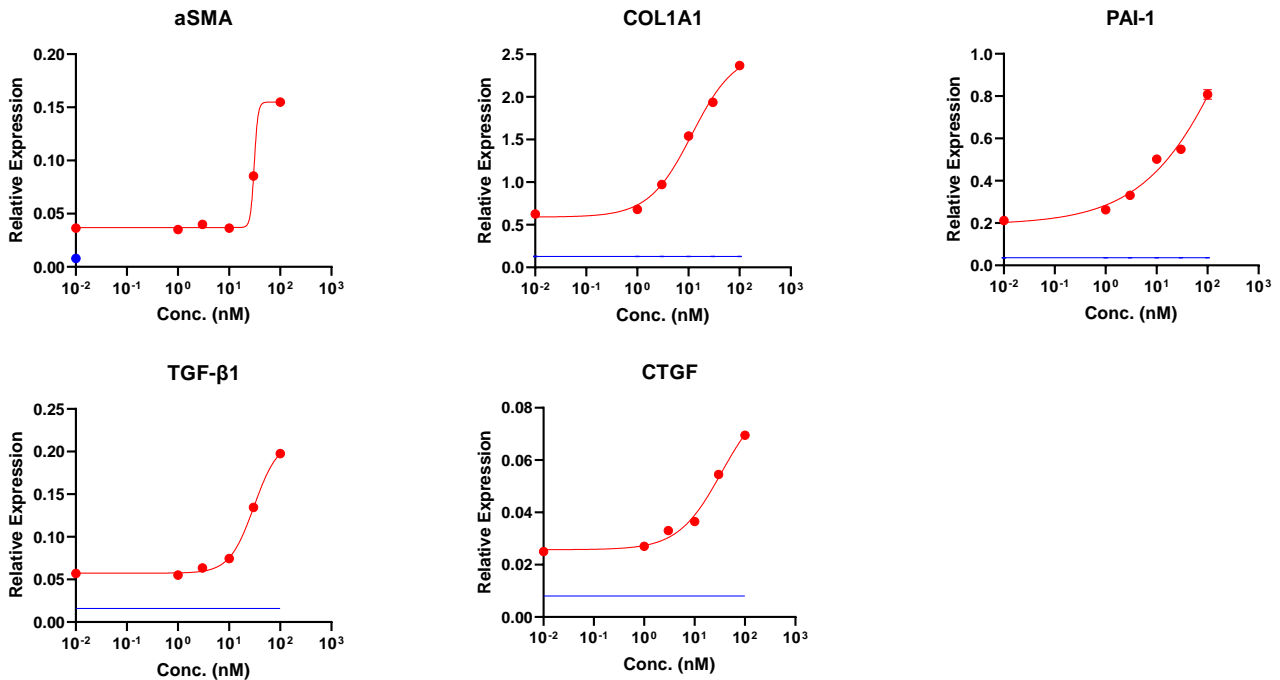
Raw Data (Ct Value):

Treatment	- TGF-β		+ TGF-β (5 ng/mL)											
	0 nM		0 nM		1 nM		3 nM		10 nM		30 nM		100 nM	
GAPDH	17.21	17.22	17.17	17.24	17.09	17.12	17.20	17.23	17.19	17.24	17.13	17.22	17.36	17.42
αSMA	24.18	24.21	21.94	22.04	21.89	21.91	21.82	21.87	21.95	21.97	20.65	20.75	20.07	20.17
COL1A1	20.16	20.23	17.84	17.89	17.63	17.72	17.21	17.23	16.58	16.58	16.19	16.23	16.12	16.19
PAI-1	21.97	21.99	19.42	19.51	19.02	19.02	18.76	18.83	18.21	18.23	17.98	18.05	17.64	17.65
TGF-β1	23.17	23.21	21.28	21.37	21.27	21.34	21.17	21.25	20.93	20.96	20.02	20.04	19.70	19.79
CTGF	24.10	24.14	22.49	22.50	22.26	22.33	22.10	22.18	21.94	22.02	21.32	21.34	21.19	21.24

Normalized value (mRNA level compared to GAPDH):

Treatment	- TGF-β		+ TGF-β (5 ng/mL)											
	0 nM		0 nM		1 nM		3 nM		10 nM		30 nM		100 nM	
GAPDH	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
αSMA	0.008	0.008	0.037	0.036	0.036	0.034	0.041	0.039	0.037	0.036	0.087	0.084	0.153	0.157
COL1A1	0.129	0.127	0.629	0.622	0.688	0.674	0.993	0.951	1.526	1.556	1.919	1.952	2.362	2.374
PAI-1	0.037	0.035	0.210	0.214	0.262	0.263	0.339	0.324	0.493	0.511	0.555	0.542	0.824	0.792
TGF-β1	0.016	0.016	0.058	0.056	0.055	0.055	0.064	0.063	0.075	0.074	0.135	0.134	0.198	0.197
CTGF	0.008	0.008	0.025	0.025	0.028	0.026	0.033	0.033	0.037	0.036	0.055	0.054	0.070	0.069

Graph:





Compound 2: TestMol\_3  
 Compounds ID: ASXXX123\_03  
 Molecular Weight: 54 kDa  
 Assay Name: Hepatic Stellate Cell Fibrosis Assay

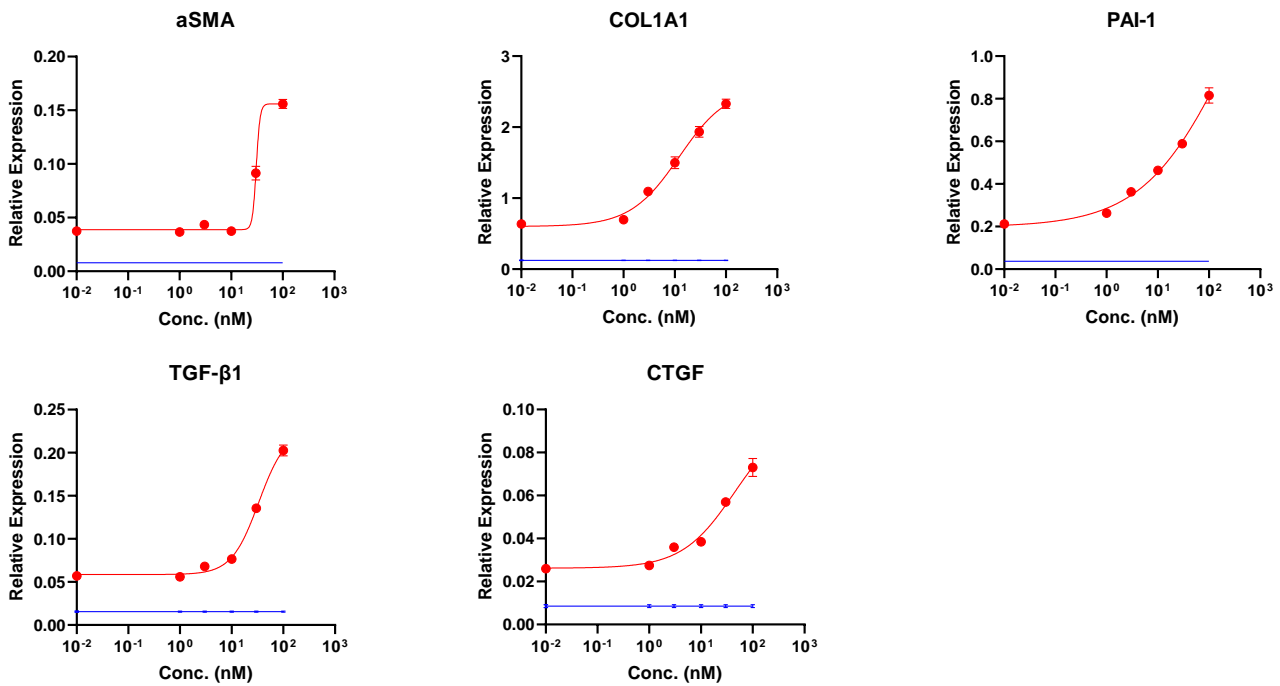
Raw Data (Ct Value):

Treatment	- TGF-β		+ TGF-β (5 ng/mL)											
	0 nM		0 nM	1 nM	3 nM	10 nM	30 nM	100 nM	0 nM	1 nM	3 nM	10 nM	30 nM	100 nM
GAPDH	17.30	17.30	17.26	17.34	17.12	17.24	17.34	17.42	17.22	17.33	17.22	17.32	17.50	17.45
αSMA	24.26	24.34	22.01	22.05	21.99	21.94	21.93	21.88	21.97	22.05	20.75	20.71	20.15	20.17
COL1A1	20.28	20.35	17.96	17.94	17.71	17.70	17.21	17.29	16.58	16.80	16.31	16.33	16.25	16.26
PAI-1	22.01	22.04	19.56	19.51	19.10	19.12	18.80	18.89	18.35	18.42	18.01	18.06	17.75	17.79
TGF-β1	23.23	23.34	21.42	21.43	21.30	21.38	21.25	21.28	20.97	20.99	20.11	20.21	19.77	19.79
CTGF	24.12	24.23	22.51	22.60	22.26	22.47	22.15	22.21	21.95	22.02	21.34	21.47	21.21	21.28

Normalized value (mRNA level compared to GAPDH):

Treatment	- TGF-β		+ TGF-β (5 ng/mL)											
	0 nM		0 nM	1 nM	3 nM	10 nM	30 nM	100 nM	0 nM	1 nM	3 nM	10 nM	30 nM	100 nM
GAPDH	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
αSMA	0.008	0.008	0.037	0.038	0.034	0.039	0.041	0.046	0.037	0.038	0.087	0.096	0.159	0.153
COL1A1	0.126	0.121	0.616	0.659	0.666	0.726	1.092	1.093	1.557	1.440	1.880	1.988	2.375	2.281
PAI-1	0.038	0.037	0.202	0.222	0.254	0.272	0.364	0.362	0.458	0.470	0.578	0.599	0.841	0.790
TGF-β1	0.016	0.015	0.056	0.058	0.055	0.057	0.067	0.069	0.074	0.079	0.136	0.135	0.207	0.198
CTGF	0.009	0.008	0.026	0.026	0.028	0.027	0.036	0.036	0.038	0.039	0.057	0.057	0.076	0.070

Graph:



## 5. Conclusion

As TestMol\_2 (ASXXX123\_02) was failed in the QC step, TestMol\_1 (ASXXX123\_01) and TestMol\_2 (ASXXX123\_03) were tested by our Hepatic Stellate Cell Fibrosis Assay. After treated with TestMol\_1 (ASXXX123\_01) and TestMol\_2 (ASXXX123\_03), the TGF- $\beta$  induced fibrosis related genes' expression ( $\alpha$ SMA, COL1A1, PAI-1, CTGF) were dose dependently further up regulated. These results indicate that these 2 compounds might have a potential function in accelerating the progression of fibrosis.



# INNOVO® Assay Sharing