STAT3 Transcription Factor Assay Kit

Item No. 601950

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GENERAL INFORMATION

Materials Supplied

Item Number	Item Name	96 wells Quantity/Size	Storage Temperature
10006880	Transcription Factor Binding Assay Buffer (4X)	1 vial/3 ml	4°C
601951	Transcription Factor STAT3 Primary Antibody	1 vial/120 µl	-20°C
601731	Transcription Factor STAT3 Positive Control	1 vial/150 µl	-80°C
601733	Transcription Factor STAT Competitor dsDNA	1 vial/120 µl	-20°C
601734	Transcription Factor STAT 96-well Strip Plate	1 plate	4°C
10007472	Transcription Factor Reagent A	1 vial/120 µl	-20°C
10006882	Transcription Factor Antibody Binding Buffer (10X)	1 vial/3 ml	4°C
400062	Wash Buffer Concentrate (400X)	1 vial/5 ml	RT
400035	Polysorbate 20	1 vial/3 ml	RT
10009279	Transcription Factor Goat Anti-Mouse HRP Conjugate	1 vial/120 µl	-20°C
400012	96-Well Cover Sheet	1 ea	RT
10006888	Transcription Factor Developing Solution	1 vial/12 ml	4°C
10006889	Transcription Factor Stop Solution	1 vial/12 ml	RT

If any of the items listed in the **Materials Supplied** on page 3 are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

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.

Precautions

Please read these instructions carefully before beginning this assay.

This kit may not perform as described if any reagent or procedure is replaced or modified.

The positive control should be thawed on ice shortly before being added to the plate. It is recommended that the positive control be stored as 50 µl aliquots at -80°C to avoid repeated free-thaw cycles.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Fax: 734-971-3640

Email: techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed in the Materials Supplied section (see page 3) and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A plate reader capable of measuring absorbance at 450 nm
2. Adjustable pipettes and a repeating pipettor
3. A source of pure water; glass-distilled, ultrapure, or HPLC-grade water is acceptable *NOTE: UltraPure Water is available for purchase from Cayman (Item No. 400000).*
4. 300 mM dithiothreitol (DTT)
5. Nuclear Extraction Kit available from Cayman (Item No. 10009277) or buffers for preparation of nuclear extracts
6. An orbital microplate shaker

NOTE: The components in each kit lot have been quality assured and warranted in this specific combinantion only; please do not mix them with components from other lots.

INTRODUCTION

Background

Signal transducers and activators of transcription (STATs) are a family of cytoplasmic transcription factors that convey signals from the cell membrane to the nucleus.[1] STAT3 is activated through phosphorylation by JAKs associated with immune-related and growth factor receptors.[1-3] Once activated, STAT3 forms homodimers, or heterodimers with other STAT family members, that are transported to the nucleus and bind to the interferon-stimulated response element (ISRE) and gamma interferon activation site (GAS) to activate transcription of cytokine-inducible genes.[1,2] STAT3 is constitutively activated in more than half of human cancers where it promotes cell proliferation, cell survival, angiogenesis, and tumor-associated immunosuppression.[1,4,5] Continuous activation of STAT3 is also required in maintaining the pluripotency of mouse embryonic stem cells.[6] Direct inhibitors of STAT3 activation and upstream inhibitors of STAT3 signaling inhibit proliferation in breast cancer cell lines, indicating the potential for STAT3 inhibitors as cancer therapeutics.[2]

About This Assay

Cayman's STAT3 Transcription Factor Assay Kit is a non-radioactive, sensitive method for detecting specific STAT3 DNA binding activity in nuclear extracts. As an alternative to the cumbersome electrophoretic mobility shift assay (EMSA) or the lower throughput ChIP assay, this ELISA is extremely easy to perform, as shown in Figure 1 on page 8. This kit is an ideal way to measure STAT3 transcriptional activity downstream of cytokine or drug treatment and manipulation of cells *in vitro* or *in vivo*. Cayman's STAT3 Transcription Factor Assay Kit detects human, mouse, rat, and non-human primate STAT3 binding on the DNA target sequence. It does not cross react with other STAT transcription factors.

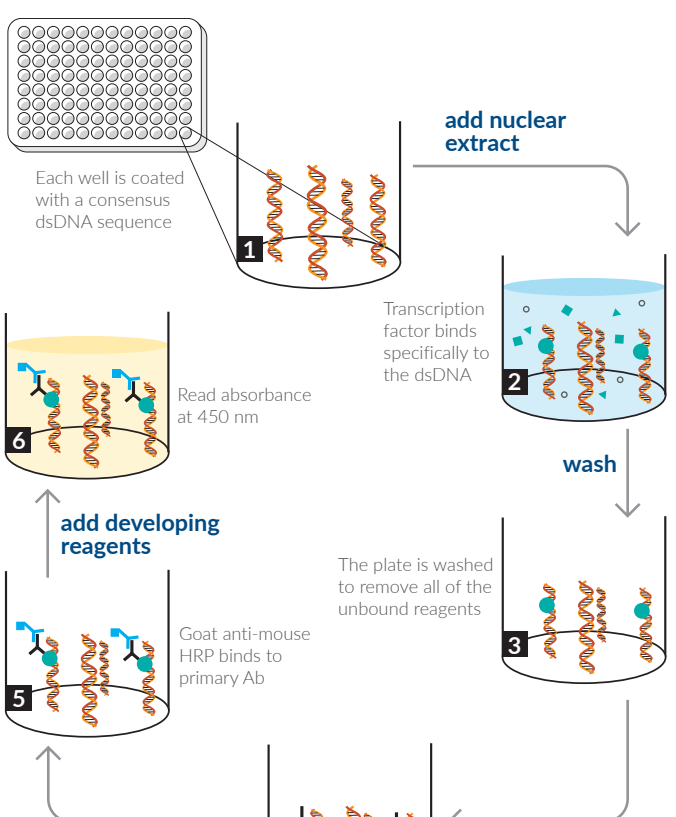

Figure 1. Schematic of the STAT3 Transcription Factor Binding Assay

PRE-ASSAY PREPARATION

Reagent Preparation

1. **Transcription Factor Antibody Binding Buffer (1X) Preparation**

   Dilute the Transcription Factor Antibody Binding Buffer (10X) (ABB; Item No. 10006882) 1:10 by adding 27 ml of pure water. Store at 4°C for up to six months.

2. **Wash Buffer (1X) Preparation**

   Dilute the Wash Buffer (400X) (Item No. 400062) to a total volume of 2 L with pure water and add 1 ml of Polysorbate 20 (Item No. 400035). Scale as necessary. Store Wash Buffer (1X) at 4°C for up to two months. *NOTE: It is normal for the concentrated buffer to contain crystalline salts. These will completely dissolve upon dilution with pure water. Polysorbate 20 is a viscous liquid and cannot be measured by a regular pipette. A positive displacement pipette or a syringe should be used to deliver small quantities accurately.*

3. **Complete Transcription Factor Binding Assay Buffer Preparation**

   Prepare 10 ml of Complete Transcription Factor Binding Assay Buffer (CTFB) by adding 2.5 ml of Transcription Factor Binding Assay Buffer (4X) (Item No. 10006880), 0.1 ml of Transcription Factor Reagent A (Item No. 10007472), and 0.1 ml of 300 mM DTT to 7.3 ml of pure water. Scale as necessary. It is recommended that the CTFB be used the same day it is prepared.

4. **Transcription Factor STAT3 Positive Control**

   Transcription Factor STAT3 Positive Control (Item No. 601731) contains 150 µl of clarified nuclear extract. This nuclear extract is provided as a positive control (PC) for STAT3 activation; it is not intended to be used as a standard for quantitative measurements. The PC provided will produce a strong signal (>0.5 AU at 450 nm) when used at 10 µl/well. Serial two-fold dilutions of this PC can be used for monitoring the dynamic range of the assay. A decrease in signal may occur with repeated freeze/thaw cycles. It is recommended that the Transcription Factor STAT3 Positive Control be stored as 50 µl aliquots at -80°C to avoid loss in signal from repeated freeze/thaw cycles.

5. **Transcription Factor STAT Competitor dsDNA**

   Transcription Factor STAT Competitor dsDNA (Item No. 601733) contains 120 µl of annealed oligonucleotides with STAT3 binding sequence. This dsDNA is provided to inhibit the binding of STAT3 in PC or samples to the DNA immobilized on the plate. A significant suppression (>50%) of PC signal will be produced by this competitor when used at 10 µl/well premixed with 10 µl of undiluted PC before adding to the well with 80 µl CTFB.

ASSAY PROTOCOL

Plate Set Up

There is no specific pattern for using the wells on the plate. A typical layout for the PC, PC plus competitor (C1), blank and unknown samples of nuclear extracts (S1-S44) to be measured in duplicate is given below in Figure 2. We suggest you record the contents of each well on the template sheet provided (see page 27).

A suggested plate format is shown in Figure 2, below. The user may vary the location and type of wells present as necessary for each particular experiment.

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1	S9	S9	S17	S17	S25	S25	S33	S33	S41	S41
B	S2	S2	S10	S10	S18	S18	S26	S26	S34	S34	S42	S42
C	S3	S3	S11	S11	S19	S19	S27	S27	S35	S35	S43	S43
D	S4	S4	S12	S12	S20	S20	S28	S28	S36	S36	S44	S44
E	S5	S5	S13	S13	S21	S21	S29	S29	S37	S37	NSB	NSB
F	S6	S6	S14	S14	S22	S22	S30	S30	S38	S38	PC	PC
G	S7	S7	S15	S15	S23	S23	S31	S31	S39	S39	Blk	Blk
H	S8	S8	S16	S16	S24	S24	S32	S32	S40	S40	C1	C1

S1-S44 - Sample Wells
NSB - Non-specific Binding Wells
PC - Positive Control Wells
Blk - Blank Wells
C1 - Competitor dsDNA Wells

Figure 2. Sample plate format

General Information

- A minimum of two Blk, two zero wells (also referred to as Non-Specific Binding (NSB)), and two PC wells should be included in each assay.
- We recommend using Cayman's Nuclear Extraction Kit (Item No. 10009277) for sample preparation.

Performing the Assay

Binding of active STAT3 to the consensus sequence:

1. Equilibrate the plate and buffers to room temperature prior to opening. Remove the plate from the foil and select the number of strips needed. The 96-well plate supplied with this kit is ready to use.

   NOTE: If you are not using all of the strips at once, place the unused strips back in the plate packet and store at 4°C. Be sure that the packet is sealed with the desiccant inside.

2. Add appropriate amount of reagent(s) listed below to the designated wells as follows:

   Blk: add 100 µl of CTFB to designated wells.

   Zero Wells: add 100 µl of CTFB to designated wells.

   PC: Add 90 µl of CTFB to each well, then add 10 µl of the undiluted PC to each well.

   S1-S44: Add 90 µl of CTFB followed by 10 µl of sample to designated wells. Competitor (optional): Add 80 µl of CTFB per designated well. In a microcentrifuge tube, premix 10 µl of Transcription Factor STAT Competitor dsDNA (Item No. 601733) with 10 µl undiluted PC, then add to the designated competitor well.

3. Use the 96-Well Cover Sheet (Item No. 400012) provided to seal the plate. Incubate overnight at 4°C without shaking or one hour at room temperature on an orbital shaker.

4. Empty the wells and wash five times with 200 µl of Wash Buffer (1X). After the final wash, tap the plate on a paper towel to remove any residual wash buffer.

Addition of Transcription Factor STAT3 Primary Antibody

5. Dilute the Transcription Factor STAT3 Primary Antibody (Item No. 601951) 1:100 in ABB (1X). Add 100 µl to each well except the Blk wells.
6. Seal the plate with the cover sheet.
7. Incubate for one hour at room temperature on an orbital shaker.
8. Empty the wells and wash five times with 200 µl of Wash Buffer (1X). After the final wash, tap the plate three to five times on a paper towel to remove any residual wash buffer.

Addition of Transcription Factor Goat Anti-Mouse HRP Conjugate

9. Dilute the Transcription Factor Goat Anti-Mouse HRP (Item No. 10009279) 1:100 in ABB (1X). Add 100 µl to each well except the Blk wells.
10. Seal the plate with the cover sheet.
11. Incubate for one hour at room temperature on an orbital shaker.
12. Empty the wells and wash five times with 200 µl of Wash Buffer (1X). After the final wash, tap the plate three to five times on a paper towel to remove any residual wash buffer.

Develop and Read the Plate

13. Add 100 µl of Transcription Factor Developing Solution (Item No. 10006888) to each well.
14. Seal the plate with the cover sheet, and incubate the plate for 30 minutes at room temperature on an orbital shaker protected from light.
15. Remove cover sheet and add 100 µl of Transcription Factor Stop Solution (Item No. 10006889) per well. The solution within the wells will change from blue to yellow.
16. Read absorbance at 450 nm within five minutes of adding the Transcription Factor Stop Solution.

Assay Procedure Summary

NOTE: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when initially performing the assay.

1. Add reagents to wells as indicated in Table 1 on page 18.
2. Incubate overnight at 4°C without shaking or one hour at room temperature on an orbital shaker.
3. Wash each well five times with 200 µl of Wash Buffer (1X).
4. Add 100 µl of Transcription Factor STAT3 Primary Antibody (1:100) per well (except Blk wells).
5. Incubate one hour at room temperature on an orbital shaker.
6. Wash each well five times with 200 µl of Wash Buffer (1X).
7. Add 100 µl of Transcription Factor Goat Anti-Mouse HRP Conjugate (1:100) (except Blk wells).
8. Incubate one hour at room temperature on an orbital shaker.
9. Wash each well five times with 200 µl of Wash Buffer (1X).
10. Add 100 µl of Transcription Factor Developing Solution per well.
11. Incubate 30 minutes at room temperature on an orbital shaker, protected from light.
12. Add 100 µl of Transcription Factor Stop Solution per well.
13. Read the absorbance at 450 nm.

Reagent	Blk	Zero Wells	PC	S1-S44	Competitor
CTFB	100 µl	100 µl	90 µl	90 µl	80 µl
Positive Control			10 µl		10 µl
Samples				10 µl	
Competitor dsDNA					10 µl

Table 1. Plate set up summary

ANALYSIS

Performance Characteristics

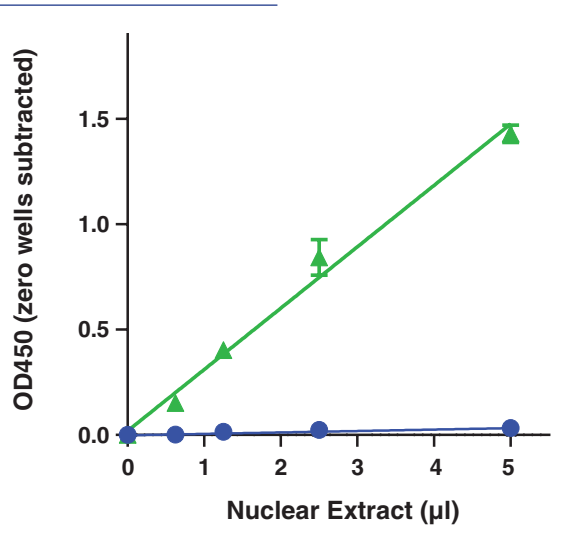

Figure 3. Example data generated using the STAT3 Transcription Factor Assay Kit. Nuclear extracts were prepared from IL-6-stimulated and unstimulated HepG2 cells. Serial dilutions of the nuclear extracts were analyzed using the STAT3 Transcription Factor Assay Kit.

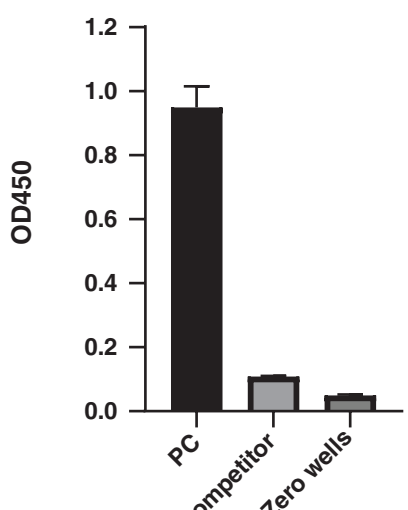

Figure 4. Typical decrease in absorbance of PC by Transcription Factor STAT Competitor dsDNA. The co-incubation of PC with competitor dsDNA drastically reduced the binding of STAT3 to the plate.

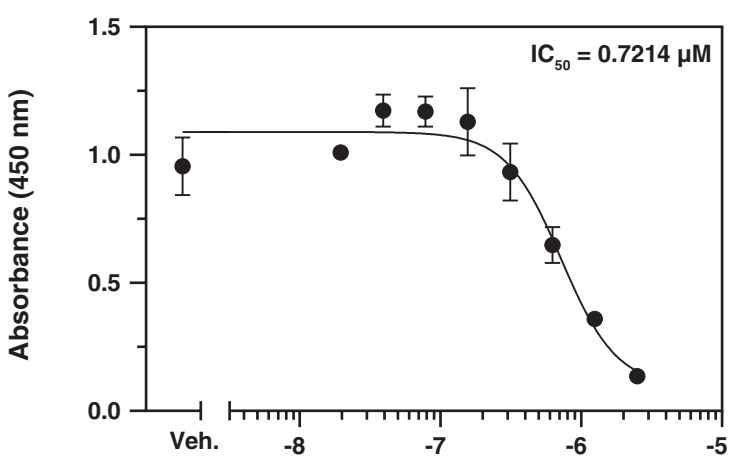

Figure 5. Inhibition of STAT3 binding by Stattic. Pre-incubation of the PC with Stattic, a small molecule inhibitor of STAT3, before adding to the Transcription Factor STAT 96-well Strip Plate exhibited dose-dependent inhibition of STAT3 binding to the plate.

Precision:

Intra-assay precision was determined by analyzing 8-12 replicates of PC, a 1:8 dilution of PC in CTFB, and the zero wells in a single assay.

Sample	PC	PC (1:8 dilution of PC in CTFB)	Zero Wells
%CV	8.6	7.8	6.7

Table 2. Intra-assay Precision

Inter-assay precision was determined by analyzing 8-12 replicates in three separate assays on different days.

Sample	PC	PC (1:8 dilution of PC in CTFB)	Zero Wells
%CV	13.5	6.7	7.3

Table 3. Inter-assay Precision

RESOURCES

Interferences

The following reagents were tested for interference in the assay.

Reagent	Will Interfere (Yes or No)
EGTA (≤1 mM)	No
EDTA (≤0.5 mM)	No
$ZnCl_2$ (any concentration)	Yes
DTT (between 1 and 5 mM)	No
Dimethylsulfoxide (≤1.5%)	No

Table 4. Interferences

Troubleshooting

Problem	Possible Causes	Recommended Solutions
No signal or weak signal in control wells	A. Omission of key reagent B. Incorrect plate reader settings C. Reagent expired D. Developing reagent used cold	A. Check that all reagents have been added and in the correct order; perform the assay using the PC B. Check wavelength setting on plate reader and change to 450 nm C. Check kit expiration date D. Prewarm the developing solution to room temperature prior to use
High signal in all wells	A. Incorrect dilution of antibody (too high) B. Improper/inadequate washing of wells C. Over-developing	A. Check antibody dilutions and use amounts outlined in instructions B. Follow the protocol for washing wells using the correct number of times and volumes C. Decrease the incubation time when using the developing reagent
High background (zero wells)	Incorrect dilution of antibody (too high)	Check antibody dilutions and use amounts outlined in the instructions
Weak signal in sample wells	A. Sample concentration too low B. Incorrect dilution of antibody C. Salt concentrations affecting binding between DNA and protein	A. Increase the amount of nuclear extract used; loss of signal can occur with multiple freeze/thaw cycles of the sample; prepare fresh nuclear extracts and aliquot B. Check antibody dilutions and use amounts outlined in the instructions C. Reduce the amount of nuclear extract used in the assay or reduce the amount of salt in the nuclear extracts (alternatively, perform buffer exchange)

References

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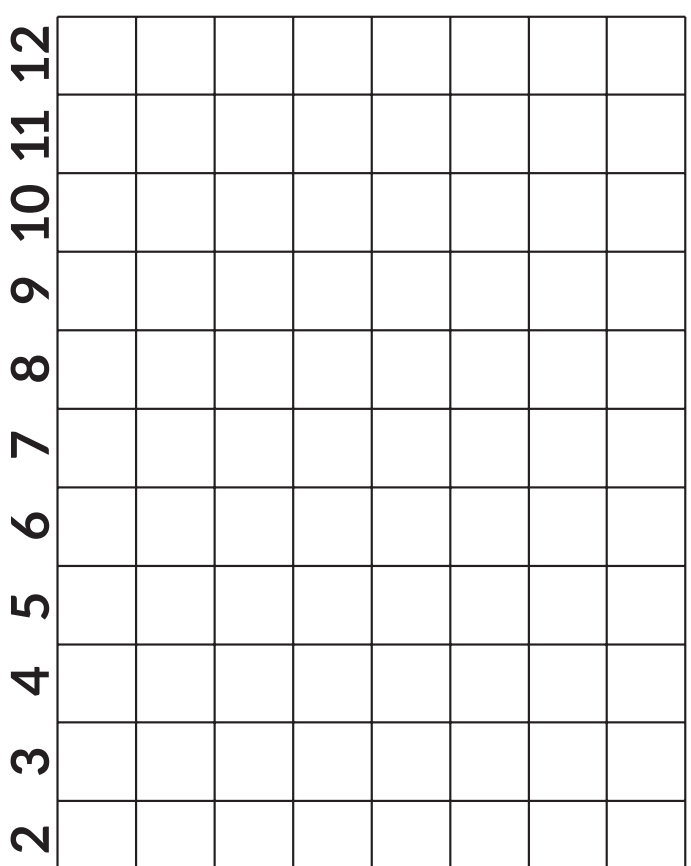
2. Ma, J., Qin, L., and Li. X. Role of STAT3 signaling pathway in breast cancer. *Cell Commun. Signal.* **18(1)**, 33 (2020).
3. Poli, V. The role of C/EBP isoforms in the control of inflammatory and native immunity functions. **273(45)**, 29279-29282 (1998).
4. Guanizo, A.C., Fernando, C.D., Garama, D.J., *et. al.* STAT3: A multifaceted oncoprotein. *Growth Factors* **36(1-2)**, 1-14 (2018).
5. Wang, Y., Shen, Y., Wang, S., *et. al.* The role of STAT3 in leading the crosstalk between human cancers and the immune system. *Cancer Lett.* **415**, 117-128 (2018).
6. Madoux, F., Koenig, M., Nelson, E., *et. al.* Modulators of STAT transcription factors for the targeted therapy of cancer (STAT3 activators). Probe Reports from the NIH Molecular Libraries Program (2009).



NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.