ab117140 – DNA-Protein Binding Assay Kit (Fluorometric)

Instructions for Use

For the measurement protein-DNA interactions in vitro, specifically for detecting transcription factor activation using mammalian tissue and cell extracts

This product is for research use only and is not intended for diagnostic use.

Version 3 Last Updated 10 December 2015

Table of Contents

INTRODUCTION

1. BACKGROUND 2
2. ASSAY SUMMARY 3

GENERAL INFORMATION

3. PRECAUTIONS 4
4. STORAGE AND STABILITY 4
5. MATERIALS SUPPLIED 5
6. MATERIALS REQUIRED, NOT SUPPLIED 5
7. LIMITATIONS 6
8. TECHNICAL HINTS 6

ASSAY PREPARATION

9. REAGENT PREPARATION 7
10. SAMPLE PREPARATION 7

ASSAY PROCEDURE

11. ASSAY PROCEDURE 8

DATA ANALYSIS

12. ANALYSIS 9

RESOURCES

13. TROUBLESHOOTING 10
14. NOTES 11

1. BACKGROUND

Protein-DNA interaction plays a critical role in cellular functions such as signal transduction, gene transcription, chromosome segregation, DNA replication and recombination, as well as epigenetic silencing. Identifying the genetic targets of DNA binding proteins and knowing the mechanisms of protein-DNA interaction is important for understanding cellular processes.
Measurement of direct interactions between protein and DNA in vitro has an advantage for analyzing binding of different transcription factors to specific DNA consensus sequences located in gene promoters. Several methods such as electrophoretic mobility shift assay (EMSA) and reporter gene assay have been developed to analyze direct interactions between protein and DNA in vitro. However, these methods available so far are time consuming, labor intensive, and have low throughput, or generate radioactive waste.

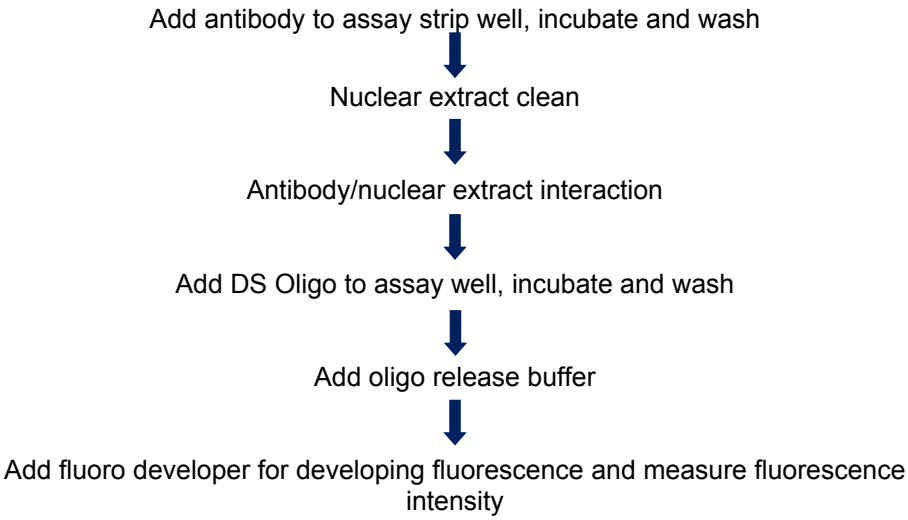
ab117140 uses a unique procedure and composition to investigate protein-DNA interaction in vitro efficiently.
This kit has the following features:

- The fastest procedure: a complete assay can be finished within 3 hours.
- Strip microplate format makes the assay flexible: manual or high throughput analysis.
- Fluorometrically qualify or quantify protein activation and use radioactive-free materials: safer to handle.
- No requirement for coating antibodies, using secondary antibodies, or for labeling oligo-nucleotides: save time and reduce labor.
- Simple, reliable, and consistent assay conditions.

The DNA-Protein Binding Assay Kit (Fluorometric) is designed for measuring the transcription factor (TF) of DNA binding activity in nuclear extracts. In this assay, target TF in nuclear extract is captured by high affinity antibodies onto the assay microwells. A double-strand

oligonucleotide (oligo), containing DNA binding consensus sequence for the target TF, is added into the microwell and bound to active TF in the binding assay buffer. The bound oligo is then fluorometrically detected.

2. ASSAY SUMMARY



3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit as given in the table upon receipt.

Observe the storage conditions for individual prepared components in sections 9 & 10.

Check if Wash Buffer contains salt precipitates before use. If so, warm at room temperature or 37°C and shake the buffer until the salts are re-dissolved.

5. MATERIALS SUPPLIED

Item	Quantity (96 tests)	Storage Condition (Before Preparation)
10X Wash Buffer	20 mL	4°C
Antibody Buffer	6 mL	RT
Extract Cleaner*	100 µL	-20°C
Assay Buffer	10 mL	RT
Oligo Release Solution	11 mL	RT
Fluoro Developer*	0.2 mL	-20°C
DTT (500X)*	22 µL	4°C
96-Well Plate	1	RT
8-Well Assay Strip (with Frame)	12	4°C

*Spin the solution down to the bottom after thawing, prior to use

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Variable temperature waterbath
- Vortex mixer
- Desktop centrifuge (up to 14,000 rpm)
- Orbital shaker
- Pipettes and pipette tips
- 1.5 ml microcentrifuge tubes
- Oligonucleotide of interest
- Primary antibody of interest

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures
- Do not use kit or components if it has exceeded the expiration date on the kit labels
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding

8. TECHNICAL HINTS

- Avoid foaming or bubbles when mixing or reconstituting components
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions
- Ensure plates are properly sealed or covered during incubation steps
- Complete removal of all solutions and buffers during wash steps
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions**

9. REAGENT PREPARATION

Prepare fresh reagents immediately prior to use.

9.1 **1X Wash Buffer**

Dilute 10X Wash Buffer with distilled water (pH 7.2 to 7.5) at a 1:10 ratio (e.g. 1 mL of 10X Wash Buffer + 9 mL of distilled water) to make 1X Wash Buffer.

9.2 **Primary Antibody**

Dilute your primary antibody (IgG type, 1:100) to 10 µg/mL with Antibody Buffer.

9.3 **Completed Assay Buffer Solution**

Add 1 µL of DTT (500X) to 500 µL of Assay Buffer.

10. SAMPLE PREPARATION

10.1 **Nuclear Extraction:** Prepare nuclear extracts from treated or untreated cells/tissues by using you own successful method. For your convenience and the best results Abcam's Nuclear Extraction Kit (ab113474) is optimized for use with this kit.

11. ASSAY PROCEDURE

11.1 Determine the number of strip wells required. Leave these strips in the plate frame (remaining unused strips can be placed back in the bag. Seal the bag tightly and store at 4°C). Then wash the strip wells once with 150 µL of the 1X Wash Buffer.

11.2 Add 50 µLl of the diluted primary antibody to the strip wells, cover the wells with Parafilm M, and incubate at room temperature for 1-1.5 hours. Meanwhile, proceed to step 11.3.

11.3 Clean nuclear extracts by adding Extract Cleaner at a 1:10 ratio to the nuclear extract and incubate for 5 minutes on ice. Centrifuge at 12,000 rpm for 2 minutes at 4°C to collect supernatant (cleaned nuclear extracts).

11.4 Aspirate antibody solution and wash each well with 150 µL of 1X Wash Buffer three times.

11.5 Add 100 µL of 1X Wash Buffer into each well, followed by adding 4-6 µL of cleaned nuclear extracts (5-20 µg) or purified transcription factor proteins (0.5-1 µg). Incubate at room temperature for 1 hour on a rocking platform (50-100 rpm). For the blank, add 4-6 µL of 1X Wash Buffer instead of nuclear extracts.

11.6 Aspirate the solution and wash each well with 150 µL of 1X Wash Buffer three times.

11.7 Add 50 µL of the Completed Assay Buffer Solution and 2 µL (150-200 ng) of your double-strand oligonucleotide (DS Oligo) to the wells. Mix and incubate at 37°C for 1 hour.

11.8 Aspirate the solution and wash each well with 150 µL of 1X Wash Buffer five times.

11.9 Add 100 µL of Oligo Release Solution to each well and incubate at 55°C for 15 minutes.

11.10 Transfer the solution to the 96-well assay plate provided. Add 2 µL of Fluoro Developer to each well and measure fluorescence on a fluorometer at Ex/Em = 495/520 nm.

12. ANALYSIS

Calculate binding activity using the following formula:

Binding Activity = Sample RFU – Blank RFU x sample dilution

13. TROUBLESHOOTING

Problem	Cause	Solution
No Signal for the Sample	The protein sample is not properly extracted.	Ensure the protein extraction protocol is suitable for nuclear protein extraction.
	The protein amount is added into the well insufficiently.	Ensure extract contains a sufficient amount of proteins.
	The sample is not prepared from fresh cells or tissues.	The nuclear extracts from frozen cells or tissues significantly lose binding activity. A fresh sample should be used.
	Nuclear extracts are incorrectly stored or have been stored for a long period of time.	Ensure the nuclear extracts are stored at –80°C for no more than 8 weeks.

High Background Present for the Blank	The well is not washed sufficiently.	Check if wash at each step is performed according to the protocol.
	Nuclear extract is not sufficiently cleaned.	Ensure extract Cleaner is added and incubated for a sufficient amount of time, as stated in step 11.3
	Insufficient antibody dilution.	Increase antibody dilution.

14. NOTES

UK, EU and ROW
Email: technical@abcam.com | Tel: +44-(0)1223-696000

Austria
Email: wissenschaftlicherdienst@abcam.com | Tel: 019-288-259

France
Email: supportscientifique@abcam.com | Tel: 01-46-94-62-96

Germany
Email: wissenschaftlicherdienst@abcam.com | Tel: 030-896-779-154

Spain
Email: soportecientifico@abcam.com | Tel: 911-146-554

Switzerland
Email: technical@abcam.com
Tel (Deutsch): 0435-016-424 | Tel (Français): 0615-000-530

US and Latin America
Email: us.technical@abcam.com | Tel: 888-77-ABCAM (22226)

Canada
Email: ca.technical@abcam.com | Tel: 877-749-8807

China and Asia Pacific
Email: hk.technical@abcam.com | Tel: 108008523689 (中國聯通)

Japan
Email: technical@abcam.co.jp | Tel: +81-(0)3-6231-0940

www.abcam.com | www.abcam.cn | www.abcam.co.jp