ab117139 – DNA-Protein Binding Assay Kit (Colorimetric)

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 7 February 2019

Table of Contents

1. BACKGROUND

Protein-DNA interaction plays a critical role in cellular functions such as signal transduction, gene transcription, chromosome segregation, DNA replication and recombination, and 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 the binding of different transcription factors to specific DNA consensus sequences located in the 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 produce radioactive waste.

ab117139 uses a unique procedure and composition to investigate protein-DNA interaction *in vitro* efficiently. This kit has the following features:

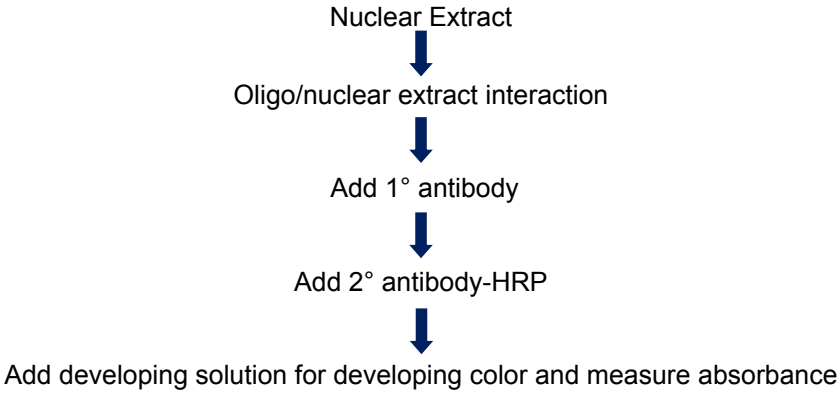
- The fastest procedure available: a complete assay can be finished within 3 hours
- Strip microplate format makes the assay flexible: manual or high throughput analysis
- Colorimetrically quantifies protein activation and uses radioactive-free materials: safer to handle
- Simple, reliable, and consistent assay conditions

ab117139 is designed for measuring the transcription factor of DNA binding activity in nuclear extracts.

In this assay, a biotin-labeled double stranded oligonucleotide (oligo), containing DNA binding consensus sequence for the target transcription factor, is incubated with nuclear extract in the binding assay buffer. Active form of transcription factor in the nuclear extract binds to its consensus

sequence. Oligo-protein complex is then captured onto the assay microwell. The target protein can be recognized with a high affinity antibody and colorimetrically measured through a detection antibody color-development reagent reaction system.

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
Assay Binding Buffer	4 mL	4°C
Antibody Dilution Buffer	20 mL	4°C
Developing Solution	10 mL	4°C
Stop Solution	6 mL	RT
8-Well Assay Strip (with Frame)	12	4°C

6. MATERIALS REQUIRED, NOT SUPPLIED

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

- Orbital shaker
- Pipettes and pipette tips
- 1.5 mL microcentrifuge tubes
- Biotinylated oligonucleotide of interest
- Primary antibody of interest
- HRP-conjugated secondary 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 to 1 µg/mL with Antibody Dilution Buffer.

9.3 **Secondary Antibody**

Dilute your HRP-conjugated Secondary Antibody to 0.5 µg/mL with 1X Wash Buffer.

10. SAMPLE PREPARATION

10.1 **Nuclear Extraction:** Prepare nuclear extracts by using you own successful method. Abcam offers a nuclear extraction kit (ab113474) 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 twice with 150 µL of the 1X Wash Buffer.

11.2 Add 23 µL of Assay Binding Buffer, 2 µL (20-40 ng) of your Biotinylated Double Stranded Oligonucleotides, and 5 µL of nuclear extracts (2-20 µg), or proteins, to each strip well. Cover the strip wells with Parafilm M and incubate at room temperature for 60 minutes. Meanwhile, set up a blank that contains biotinylated oligonucleotides without nuclear extract.

Note: *For determining specificity, add 2 µL of unlabeled oligonucleotides, 50-100 ng, to a reaction containing 2 µL of biotinylated oligonucleotides, and 5 µL of nuclear extract.*

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

11.4 Add 50 µL of the diluted primary antibody to each strip well and incubate at room temperature for 60 minutes on an orbital shaker (50-100 rpm).

11.5 Aspirate and wash each well with 150 µL of 1X Wash Buffer four times.

11.6 Add 50 µL of the diluted secondary antibody to each strip well and incubate at room temperature for 30 minutes.

11.7 Aspirate and wash each well with 150 µL of 1X Wash Buffer four times. Allow 2 minutes for last wash.

11.8 Add 100 µL of Developing Solution to each well and incubate at room temperature for 2-10 minutes away from light. Monitor the color development in the sample and standard wells (blue).

11.9 Add 50 µL of Stop Solution to each well and read absorbance on a microplate reader at 450 nm.

12. ANALYSIS

Calculate binding activity using the following formula:

Binding Activity = (Sample OD – Blank OD) 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 well insufficiently.	Ensure extract contains a sufficient amount of protein.
	The sample is not prepared from fresh cells or tissues.	The nuclear extracts from frozen cells or tissue significantly lose enzyme activity. A fresh sample should be used.
	Nuclear extracts are incorrectly stored or have been stored for a long time.	Ensure the nuclear extracts are correctly 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.
	Insufficient antibody dilution.	Increase antibody dilution.
	Overdevelopment.	Decrease development time in step 11.8.

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: 400 921 0189 / +86 21 2070 0500

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

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