

EpiQuik™ General Protein-DNA Binding Assay Kit (Colorimetric)

Base Catalog # P-2004

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

The EpiQuik™ General Protein-DNA Binding Assay Kit (Colorimetric) is suitable for analyzing protein-DNA interactions *in vitro*, specifically for detecting transcription factor activation using mammalian tissue and cell extracts.

The EpiQuik™ General Protein-DNA Binding Assay Kit (Colorimetric) offers a flexible choice of oligonucleotides and antibodies. Like using other protein-binding assay kits, if you use the EpiQuik™ General Protein-DNA Binding Assay Kit (Colorimetric), choice of a good antibody is required for capturing the protein/DNA complexes.

KIT CONTENTS

Components	96 assays P-2004-96
PD1 (10X Wash Buffer)	20 ml
PD2 (Assay Binding Buffer)	4 ml
PD3 (Antibody Dilution Buffer)	20 ml
PD4 (Developing Solution)	10 ml
PD5 (Stop Solution)	6 ml
8-Well Assay Strips (with frame)	12

SHIPPING & STORAGE

Upon receipt, store **PD1**, **PD2**, **PD3**, and **PD4** at 4°C. Store **PD5** at room temperature away from light. The kit is stable for up to 6 months from the date of shipment, when stored properly.

Note: Check if wash buffer, **PD1**, contains salt precipitates before using. If so, warm (at room temperature or 37°C) and shake the buffer until the salts are re-dissolved.

MATERIALS REQUIRED BUT NOT SUPPLIED

- ☐ Orbital shaker
- ☐ Pipettes and pipette tips
- ☐ 1.5 ml microcentrifuge tubes
- ☐ Biotinylated oligonucleotide of interest
- ☐ Primary antibody of interest
- ☐ Secondary antibody

GENERAL PRODUCT INFORMATION

Quality Control: EpigenTek guarantees the performance of all products in the manner described in our product instructions.

Product Updates: EpigenTek reserves the right to change or modify any product to enhance its performance and design. The information in this User Guide is subject to change at any time without notice. Be sure to use the latest User Guide for this kit which can be accessed online at www.epigentek.com/datasheet.

Usage Limitation: The *EpiQuik*™ kits are for research use only and are not intended for diagnostic or therapeutic application.

Intellectual Property: *EpiQuik*™ is a trademark of EpigenTek, Inc.

A BRIEF OVERVIEW

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.

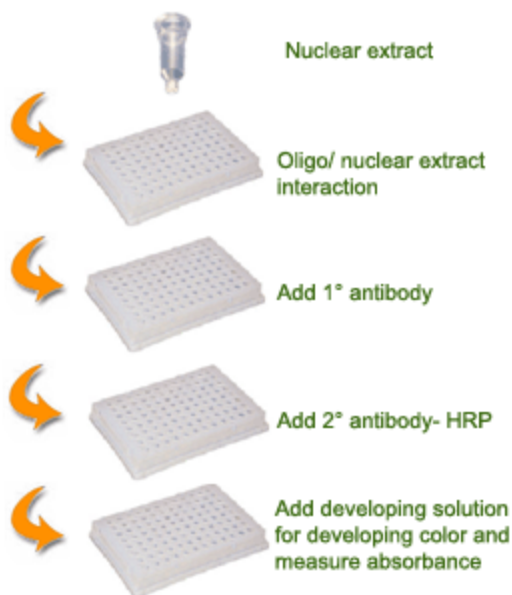
Measurement of direct interactions between protein and DNA *in vitro* has an advantage for analyzing the binding of different transcription factors to specific DNA consensus sequences located in the gene promoters. Several methods such as electrophoretic mobility shift assay (EMSA) and reporter gene 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.

The *EpiQuik*™ General Protein-DNA Binding Assay Kit (Colorimetric) 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.

PRINCIPLE & PROCEDURE

The *EpiQuik*™ General Protein-DNA Binding Assay Kit (Colorimetric) 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.



Schematic Procedure for Using the EpiQuik™ General Protein-DNA Binding Assay Kit (Colorimetric)

PROTOCOL

1. Prepare nuclear extract by using your own successful method or commercial kits. For your convenience and the best results, EpigenTek offers a nuclear extraction kit (Cat. No. OP-0002-1), optimized for use with the EpiQuik™ series.
2. 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). Dilute **PD1** 10X Wash Buffer with distilled water (pH 7.2-7.5) at a 1:10 ratio (e.g., 1 ml of **PD1** + 9 ml of distilled water). Then wash the strip wells twice with 150 μ l of the **diluted PD1**.
3. Add 23 μ l of **PD2**, 2 μ l (20-40 ng) of your Biotinylated Double Stranded Oligonucleotides, and 5 μ l of nuclear extracts (2-20 μ g), 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. (For determining specificity, add 2 μ l of unlabeled oligonucleotides, 50-100 ng, to a reaction containing 2 μ l of biotinylated oligonucleotides, and 5 μ l of nuclear extract.)
4. Aspirate and wash each well with 150 μ l of **diluted PD1** three times.
5. Dilute your primary antibody at 1 μ g/ml with **PD3**. Add 50 μ l of the *diluted primary antibody* to each strip well and incubate at room temperature for 60 minutes on an orbital shaker (50-100 rpm).
6. Aspirate and wash each well with 150 μ l of **diluted PD1** four times.

7. Dilute your HRP-conjugated Secondary Antibody at 0.5 µg/ml with **diluted PD1**. Add 50 µl of the *diluted secondary antibody* to each strip well and incubate at room temperature for 30 minutes.
8. Aspirate and wash each well with 150 µl of **diluted PD1** four times. Allow 2 minutes for last wash.
9. Add 100 µl of **PD4** to each well and incubate at room temperature for 2-10 minutes away from light. Monitor the color development in the sample wells (blue).
10. Add 50 µl of **PD5** to each well and read absorbance on a microplate reader at 450 nm.
11. Calculate binding activity using the following formula:

$$\text{Binding Activity} = \text{OD (sample - blank)} \times \text{sample dilution}$$

TROUBLESHOOTING

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 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.

Insufficient antibody dilution.

Increase antibody dilution.

Overdevelopment.

Decrease development time in step 9.

RELATED PRODUCTS

P-2005 EpiQuik™ General Protein-DNA Binding Assay Kit (Fluorometric)