ab46510 – Human DR4 ELISA Kit (CD261)

Instructions for Use

For the quantitative measurement of Human DR4 (CD261) in serum, plasma, buffered solutions and cell culture media.

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

Version 2a Last Updated 2 February 2021

Table of Contents

INTRODUCTION

1. BACKGROUND 2
2. ASSAY SUMMARY 3

GENERAL INFORMATION

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

ASSAY PREPARATION

9. REAGENT PREPARATION 8
10. STANDARD PREPARATION 10
11. SAMPLE PREPARATION AND STORAGE 12
12. PLATE PREPARATION 12

ASSAY PROCEDURE

13. ASSAY PROCEDURE 13

DATA ANALYSIS

14. CALCULATIONS 15
15. TYPICAL DATA 16
16. TYPICAL SAMPLE VALUES 17
17. ASSAY SPECIFICITY 18

RESOURCES

18. TROUBLESHOOTING 18
19. NOTES 19

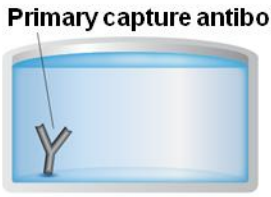
1. BACKGROUND

Abcam's DR4 (CD261) Human *in vitro* ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of DR4 in serum, plasma, buffered solutions and cell culture media.

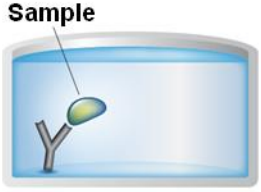
A monoclonal antibody specific for DR4 has been coated onto the wells of the microtiter strips provided. Samples, including standards of known DR4 concentrations, control specimens or unknowns are pipetted into these wells. During the first incubation, the standards or samples and a biotinylated monoclonal antibody specific for DR4 are simultaneously incubated. After washing, the enzyme Streptavidin-HRP, that binds the biotinylated antibody is added, incubated and washed. A TMB substrate solution is added which acts on the bound enzyme to induce a colored reaction product. The intensity of this colored product is directly proportional to the concentration of DR4 present in the samples.

This kit will recognize both endogenous and recombinant Human DR4.

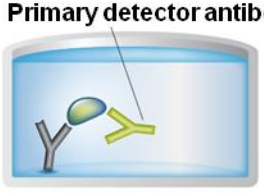
2. ASSAY SUMMARY



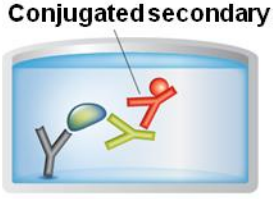
Remove appropriate number of antibody coated well strips. Equilibrate all reagents to room temperature. Prepare all the reagents, samples, and standards as instructed.



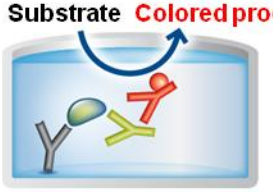
Add standard or sample to each well used. Incubate at room temperature



Aspirate and wash each well. Add prepared Biotinylated labeled detector antibody. Incubate at room temperature



Aspirate and wash each well. Add prepared Streptavidin-HRP mix to each well. Incubate at room temperature



Aspirate and wash each well. Add the TMB Solution to each well until color develops and then add the Stop Solution. Immediately begin recording the color development

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 at +2-8ºC immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 9. Reagent Preparation.

5. MATERIALS SUPPLIED

Item	Quantity		Storage Condition (Before Preparation)
	1 x 96 tests	2 x 96 tests	
DR4 Microplate (12 x 8 well strips)	96 wells	2 x 96 wells	+2-8ºC
DR4 Standard (Lyophilized)	2 vials	4 vials	+2-8ºC
10X Standard Diluent Buffer	15 mL	25 mL	+2-8ºC
Biotinylated anti-DR4	400 µL	2 x 400 µL	+2-8ºC
Biotinylated Antibody Diluent	7 mL	13 mL	+2-8ºC
Streptavidin-HRP	2 x 5 µL	4 x 5 µL	+2-8ºC
HRP Diluent	12 mL	23 mL	+2-8ºC
200X Wash Buffer	10 mL	2 x 10 mL	+2-8ºC
Chromogen TMB Substrate Solution	11 mL	24 mL	+2-8ºC
Stop Reagent	11 mL	2 x 11 mL	+2-8ºC

6. MATERIALS REQUIRED, NOT SUPPLIED

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

- Microplate reader capable of measuring absorbance at 450 nm.
- Precision pipettes to deliver 2 µL to 1 mL volumes.
- Adjustable 1-25 mL pipettes for reagent preparation.
- 100 mL and 1 liter graduated cylinders.
- Absorbent paper.
- Distilled or deionized water.
- Tubes to prepare standard or sample dilutions.
- Log-log graph paper or computer and software for ELISA data analysis.

7. LIMITATIONS

- 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.
- Since exact conditions may vary from assay to assay, a standard curve must be established for every assay performed.
- Bacterial or fungal contamination of either samples or reagents or cross-contamination between reagents may cause erroneous results.
- Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.
- Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Completely empty wells before dispensing fresh 1X Wash Buffer. Do not allow wells to sit uncovered or dry for extended periods

8. TECHNICAL HINTS

- Kit components should be stored as indicated. All the reagents should be equilibrated to room temperature before use. Reconstituted standards should be discarded after use.
- Once the desired number of strips has been removed, immediately reseal the bag to protect the remaining strips from degradation.
- Use a clean disposable plastic pipette tip for each reagent, standard, or specimen addition in order to avoid cross-contamination; for the dispensing of the Stop Solution and substrate solution, avoid pipettes with metal parts.
- Thoroughly mix the reagents and samples before use by agitation or swirling.
- All residual washing liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into the wells.
- The TMB solution is light sensitive. Avoid prolonged exposure to light. Also, avoid contact of the TMB solution with metal to prevent color development. Warning TMB is toxic avoid direct contact with hands. Dispose off properly.
- If a dark blue color develops within a few minutes after preparation, this indicates that the TMB solution has been contaminated and must be discarded. Read absorbances within 1 hour after completion of the assay.
- When pipetting reagents, maintain a consistent order of addition from well-to-well. This will ensure equal incubation times for all wells.
- Dispense the TMB solution within 15 minutes following the washing of the microtiter plate.

- **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

Equilibrate all reagents and samples to room temperature (18-25°C) prior to use.

9.1 1X Standard Diluent Buffer

Dilute the 10X Standard Diluent Buffer 10-fold in distilled water before use.

9.2 1X Wash Buffer

Dilute the 200X Wash Buffer Concentrate 200-fold in distilled water before use. Mix gently to avoid foaming. The 1X Wash Buffer can be prepared as needed according to the following table:

Number of well strips used	Volume of 200X Wash Buffer Concentrate (mL)	Volume of distilled water (mL)
1-6	5	995
1-12	10	1,990

9.3 1X Biotinylated anti-DR4

Prepare the 1X Biotinylated anti-DR4 immediately prior to use. According to the table below, dilute the Biotinylated anti-DR4 with the Biotinylated Antibody Diluent based on the number of wells being used in the assay procedure:

Number of well strips used	Volume of Biotinylated anti-DR4 µL)	Volume of Biotinylated Antibody Diluent (µL)
2	40	1,060
3	60	1,590
4	80	2,120
6	120	3,180
12	240	6,360

9.4 1X Streptavidin-HRP Solution

Add 500 µL of HRP-Diluent to the Streptavidin-HRP vial prior to use to create a Streptavidin-HRP Concentrate. Do not keep this solution for further experiments.

Subsequently, prior to use in the assay procedure, prepare the 1X Streptavidin-HRP Solution by further diluting the Streptavidin-HRP Concentrate with HRP-Diluent. Use the table below to determine the volumes of each solution required to prepare the final 1X Streptavidin-HRP Solution:

Number of well strips used	Volume of Streptavidin-HRP (µL)	Volume of HRP-Diluent (mL)
2	30	2
3	45	3
4	60	4
6	75	5
12	150	10

10. STANDARD PREPARATION

Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every use.

10.1 Prepare a 1,000 pg/mL **Standard #1** by reconstituting with the volume indicated on the vial using the 1X Standard Diluent Buffer.

10.2 Label tubes #2-6 and add 100 µL of 1X Standard Diluent Buffer.

10.3 Prepare **Standard #2** by adding 100 µL of Standard #1 to tube #2 and mix thoroughly**.**

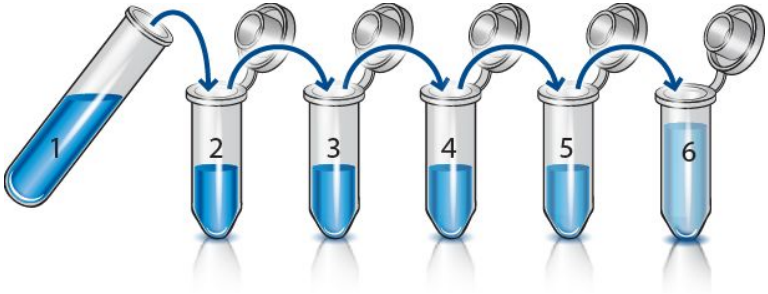
10.4 Prepare **Standard #3** by adding 100 µL of Standard #2 to tube #3 and mix thoroughly**.**

10.5 Using the table below as a guide, prepare further serial dilutions.

10.6 1X Standard Diluent Buffer serves as the zero standard (0 pg/mL).

Standard Dilution Preparation Table

Standard #	Volume to Dilute (µL)	Diluent (µL)	Total Volume (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	-	-	-	1,000	1,000
2	100	100	200	20	500
3	100	100	200	10	250
4	100	100	200	5	125
5	100	100	200	2.5	62.5
6	100	100	200	1.25	31.25



11. SAMPLE PREPARATION AND STORAGE

- **Preparation of Plasma Samples**

  Collect plasma using citrate, EDTA or heparin. Centrifuge samples at 1,000 x g for 30 minutes. Dilute samples 1:2 into 1X Standard Diluent Buffer. Store un-diluted plasma samples at -20ºC or below for up to 3 months. Avoid repeated freeze-thaw cycles.

- **Preparation of Serum Samples**

  Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 1,000 x g for 10 minutes and collect serum. Dilute samples 1:2 into 1X Standard Diluent Buffer. Store un-diluted serum at -20ºC or below. Avoid repeated freeze-thaw cycles.

- **Preparation of Cell culture Supernatants**

  Centrifuge cell culture media at 1,000 x g for 10 minutes to remove debris. Collect supernatants and assay. Store samples at -20°C or below. Avoid repeated freeze-thaw cycles.

12. PLATE PREPARATION

- The 96 well plate strips included with this kit is supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused well strips should be returned to the plate packet and stored at 4°C.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Well effects have not been observed with this assay. Contents of each well can be recorded on the template sheet included in the Resources section.

13. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**

13.1 Prior to use, mix all reagents thoroughly taking care not to create any foam within the vials.

13.2 Determine the number of microplate strips required to test the desired number of samples, plus appropriate number of wells needed for controls and standards. Remove sufficient microplate strips from the pouch.

13.3 Add 100 µL of each standard (see Section 10), including blank controls to the appropriate wells.

13.4 Add 100 µL of sample to the appropriate wells.

13.5 Cover and incubate for 1 hours at room temperature (18-25°C).

13.6 Remove the cover and wash the plate as follows:

13.6.1 Aspirate the liquid from each well.

13.6.2 Add 300 µL of 1X Wash Buffer into each well

13.6.3 Aspirate the liquid from each well.

13.6.4 Repeat for a total of 3 washes.

13.7 Add 50 µL of 1X Biotinylated anti-DR4 to all wells (see Section 9).

13.8 Cover and incubate for 1 hours at room temperature (18-25°C).

13.9 Wash as described in Step 13.6.

13.10 Add 100 µL of 1X Streptavidin-HRP solution into all wells, including the blank wells. Re-cover and incubate at room temperature for 30 minutes.

13.11 Wash as described in Step 13.6.

13.12 Add 100 µL of Chromogen TMB substrate solution into each well and incubate in the dark for 10-20 minutes at

room temperature. Avoid direct exposure to light by wrapping the plate in aluminum foil. *Note:* Incubation time of the substrate solution is usually determined by the microplate reader performances: many microplate readers record absorbance only up to 2.0 O.D. The O.D. values of the plate should be monitored and the substrate reaction stopped before positive wells are no longer accurately readable (maximum ~20 minutes).

13.13 Add 100 µL of Stop Reagent into each well. Results must be taken immediately after the addition of Stop Reagent, or within one hour, if the microplate is stored at 2-8°C in the dark.

13.14 Read absorbance of each well on a spectrophotometer using 450 nm as the primary wavelength and optionally 620 nm (610 nm to 650 nm is acceptable) as the reference wavelength.

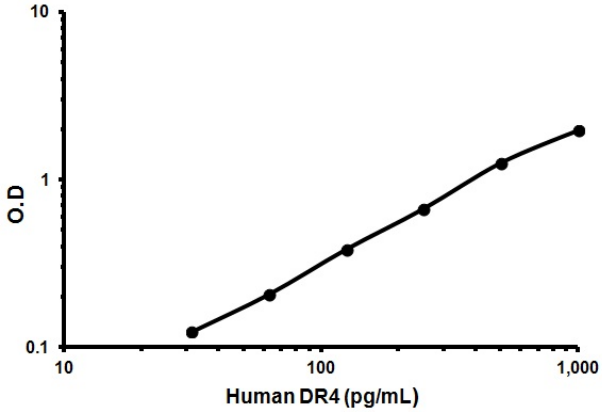
14. CALCULATIONS

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

For samples that have been diluted, the concentration read from the standard curve has to be multiplied by the dilution factor to determine the actual concentration of the target protein present.

15. TYPICAL DATA

TYPICAL STANDARD CURVE - Data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.



Conc. (pg/mL)	O.D.
1,000	1.961
500	1.255
250	0.670
125	0.385
62.5	0.208
31.25	0.124
0	0.016

16. TYPICAL SAMPLE VALUES

EXPECTED SERUM VALUES

DR4 is not detected in healthy donor sera (64 sera tested).

SENSITIVITY -

The sensitivity, minimum detectable dose of Human DR4 using this Abcam DR4 ELISA kit was found to be <8 pg/mL. This was determined by adding 3 standard deviations to the mean OD obtained when the zero standard was assayed 32 times.

PRECISION –Sample A

	Intra-Assay	Inter-Assay
n=	8	12
Mean (pg/mL)	475	498
SD	13	40
CV (%)	2.76	8.11

PRECISION –Sample B

	Intra-Assay	Inter-Assay
n=	8	12
Mean (pg/mL)	54	53
SD	4	7
CV (%)	7.57	13.87

DILUTION PARALLELISM -

One Human serum containing 1000 pg/mL of DR4 was serially diluted in standard Diluent Buffer. Linear regression of samples versus the expected concentration yielded a correlation coefficient of 0.99.

SPIKE RECOVERY -

The spike recovery was evaluated by spiking natural DR4 in Human serum. Recoveries ranged from 81% to 113% with an overall mean recovery of 93.4%.

17. ASSAY SPECIFICITY

The assay recognizes both natural and recombinant Human DR4. To define specificity of this ELISA, several proteins were tested for cross reactivity. There was no cross reactivity observed for any protein tested : TRAIL, CD117, IL-6R, IL-2R, CD116, TRAIL R2, TRAIL R3, TRAIL R4, CD178 and Granzyme B.

18. TROUBLESHOOTING

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standards dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; change to overnight standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store the reconstituted protein at -80°C, all other assay components 4°C. Keep substrate solution protected from light.

19. NOTES

For all technical and commercial enquires please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)