Enzyme Immunoassay for
Recombinant (Therapeutic) Human IgG4

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INTRODUCTION

Recombinant human monoclonal antibodies have become a major focus of new drug discovery in the Pharmaceutical and Biotech industries, with hundreds of new human monoclonal antibodies currently in preclinical development. Fully human recombinant antibodies have gradually replaced humanized mouse or rat antibodies, with IgG1 and IgG4 as the preferred engineered isotypes[2]. As part of the developmental process, human therapeutic antibody drug candidate molecules must be tested in non-human primate species to assess their pharmacokinetic profile and potential toxicity[3]. Primate and human antibodies are highly homologous, making it difficult to distinguish the human antibody drug candidate from the endogenous primate antibodies in a serum or plasma sample by traditional human IgG-specific immunoassays. Therefore, we developed two highly sensitive ELISAs that distinguish human IgG1 or human IgG4 from rhesus or cynomolgus monkey immunoglobulins. This Recombinant (Therapeutic) Human IgG4 ELISA can be used to accurately quantify human IgG4 in the serum or plasma of rhesus or cynomolgus monkeys without cross reacting with antibodies of those species. This same ELISA can also be used to quantify human IgG4 in the plasma of other preclinical test species (mouse, rat, rabbit, guinea pig, etc.) if desired. This assay is supplied with a recombinant human IgG4 kappa standard. The customer may wish to substitute their own specific drug candidate IgG4 for this standard in the assay.

PRINCIPLES OF PROCEDURE

This is a sandwich enzyme-linked immunosorbent assay (ELISA). The plate is pre-coated with anti-Human IgG Antibody and blocked, ready for the addition of samples and standards. The assay should take approximately 3 hours to run, plus any required sample preparation time.

MATERIALS PROVIDED

Component	Description	Volume	Storage	Cat no.
Anti-Human IgG Plate	96-well plate coated and blocked	1 plate	4ºC	NF04a
Assay Buffer	Buffer to dilute kit components and samples	100mL	4ºC	NF04b
10x Wash Buffer	Buffer used to wash the plate	30mL	4ºC	NF04c
Human IgG4 Standard	Lyophilized Recombinant Human IgG4	0.2µg	4ºC	NF04d
10x Detection Antibody	Anti-Human IgG4-HRP Conjugate	1.5mL	4ºC	NF04e
TMB Substrate	Stabilized TMB color reagent	15mL	4ºC	NF04f

MATERIALS NEEDED BUT NOT PROVIDED

1. Microplate reader with 450 nm filter
2. Adjustable micropipettes (10 – 1000 µL) and tips
3. 3N Sulfuric Acid (H_2SO_4)
4. Plate shaker
5. Plate cover or plastic film

STORAGE

1. Store the components of this kit at the temperatures specified on the labels.
2. Unopened reagents are stable until the indicated kit expiration date.
3. Reconstituted Standard may be used up to 1 month after reconstitution if stored at 4ºC
4. Diluted Detection Antibody must be used within 2 hours of dilution

WARNINGS AND PRECAUTIONS

1. Use aseptic technique when opening and dispensing reagents.
2. This kit is designed to work properly as provided and instructed. Additions, deletions or substitutions to the procedure or reagents are not recommended, as they may be detrimental to the assay.

PROCEDURAL NOTES

1. To minimize errors in absorbance measurements due to handling, wipe the exterior bottom of the microplate wells with a lint-free paper towel prior to inserting into the plate reader.

SAMPLE PREPARATION

If assessing human IgG4 in monkey plasma or serum, dilute the serum at least 1:5 into Assay Buffer before testing. Undiluted monkey plasma or serum contains contaminants that interfere with the accurate functioning of this ELISA.

REAGENT PREPARATION

1. **Human IgG4 Standard:** Resuspend lyophilized standard vial by adding 0.2mL of Assay Buffer directly to vial, gently invert to suspend completely, to create 1µg/mL stock solution.
2. **10x Wash Buffer:** Dilute the wash buffer 1:10 by adding 30mL of 10x Wash Buffer to 270mL of dH_2O.
3. **10x Detection Antibody:** Immediately prior to use, dilute detection antibody 1:10 by adding 1.2mL 10x Detection Antibody to 10.8mL Assay Buffer. Use within 2 hours of diluting.

STANDARD CURVE PREPARATION

Set up for the standard curve by labeling dilution tubes and dispensing the indicated volumes of Assay Buffer and 1µg/mL Standard Stock Solution according to Table 1 below.

Table 1: Standard Curve Preparation

Standard	IgG4 Concentration (ng/mL)	Assay Buffer (µL)	Transfer Volume (µL)	Transfer Source	Final Volume (µL)
S_7	50	950	50	1µg/mL Stock	500
S_6	25	500	500	S_7	500
S_5	12.5	500	500	S_6	500
S_4	6.25	500	500	S_5	500
S_3	3.13	500	500	S_4	500
S_2	1.56	500	500	S_3	500
S_1	0.781	500	500	S_2	1000
S_0	0	1000	-	-	1000

ASSAY PROCEDURE

1. Add 100 µL of Standards and Samples to the corresponding wells on the microplate in duplicate. Incubate at room temperature for 1.5 hours on an orbital plate shaker. See Scheme 1 below for a suggested plate layout.
2. Dump the contents of the plate and wash each well three times with 300 µL of Wash Buffer. After the final wash, tap the plate upside-down on a lint-free paper towel to make sure there is no solution left in the wells.
3. Add 100 µL of the Detection Antibody to each well. Incubate at room temperature for 1 hour on orbital plate shaker.
4. Wash the plate as in step 2.
5. Add 100 µL of TMB Substrate to each well. Allow the color to develop for 10 minutes at room temperature.
6. Stop the reaction by adding 100 µL per well of 3N Sulfuric Acid (H_2SO_4).
7. Read the plate at 450 nm in a microplate reader within 15 minutes.

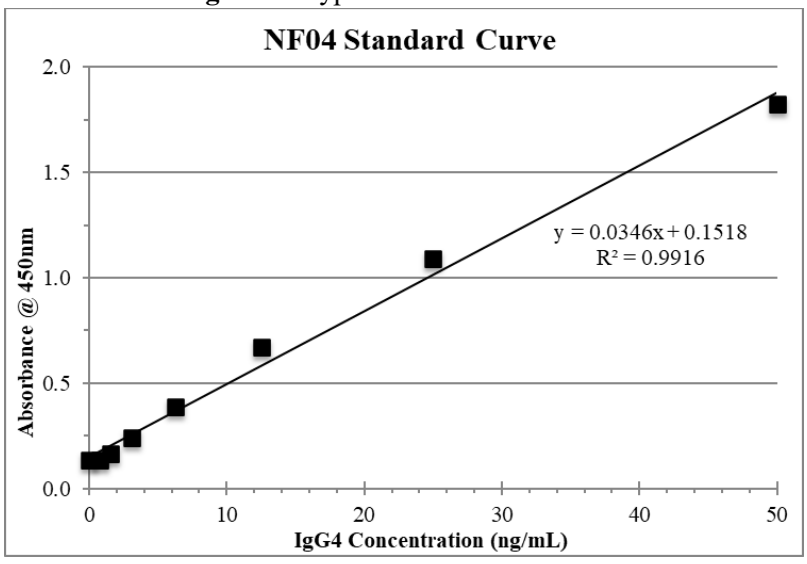
Scheme 1: Suggested Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	S_0	S_0	U_1	U_1	U_9	U_9	U_{17}	U_{17}	U_{25}	U_{25}	U_{33}	U_{33}
B	S_1	S_1	U_2	U_2	U_{10}	U_{10}	U_{18}	U_{18}	U_{26}	U_{26}	U_{34}	U_{34}
C	S_2	S_2	U_3	U_3	U_{11}	U_{11}	U_{19}	U_{19}	U_{27}	U_{27}	U_{35}	U_{35}
D	S_3	S_3	U_4	U_4	U_{12}	U_{12}	U_{20}	U_{20}	U_{28}	U_{28}	U_{36}	U_{36}
E	S_4	S_4	U_5	U_5	U_{13}	U_{13}	U_{21}	U_{21}	U_{29}	U_{29}	U_{37}	U_{37}
F	S_5	S_5	U_6	U_6	U_{14}	U_{14}	U_{22}	U_{22}	U_{30}	U_{30}	U_{38}	U_{38}
G	S_6	S_6	U_7	U_7	U_{15}	U_{15}	U_{23}	U_{23}	U_{31}	U_{31}	U_{39}	U_{39}
H	S_7	S_7	U_8	U_8	U_{16}	U_{16}	U_{24}	U_{24}	U_{32}	U_{32}	U_{40}	U_{40}

CALCULATIONS

a. Average all duplicate well absorbance values.
b. Plot a standard curve using the absorbance values of each Standard (y-axis) versus the Standard concentration (x-axis).
c. Determine the concentration of each unknown using the equation of the line.

Figure 1: Typical Standard Curve



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