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KRIBIOLISA
SP 2/0 HCP ELISA

REF: KBBP05
Ver 3.0

RUO

Immunoassay for the quantitative estimation of total SP 2/0 Host Cell Proteins.

RUO	**For Research Use Only**	REF	**Catalog Number**
	Store At	LOT	**Batch Code**
	Manufactured By		**Biological Risk**
	Expiry Date		**Consult Operating Instructions**

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Introduction:
Recombinant expression by the hybridoma cell line SP2/0 is a relatively simple and cost effective method for production of monoclonal antibodies intended for use as therapeutic agents in humans.

Intended Use:
This generic kit is intended in determining the presence of SP2/0 Host Cell Proteins contamination in various products that are manufactured through recombinant expression in SP2/0 cells. The kit has been validated successfully for testing of in process and final product HCPs in variety of products regardless of growth and purification process.

Principle:
This assay is based on the Sandwich ELISA procedure. Microwells are coated with anti- SP2/0 antibody. Addition of Anti- SP2/0:HRP Conjugate and standards or samples containing SP2/0 HCPs will form a immune complex with the coated anti- SP2/0 antibody in microwell. A washing cycle will remove free Anti- SP2/0:HRP Conjugate from the reaction well. Addition of TMB Substrate will produce blue color in microwells conataining immune complex. Addition of stop solution will terminate the enzymatic reaction and blue color will be converted to yellow color which is directly proportional to the concentration of SP2/0 HCPs present and absorbance value is measured at 450nm.

Materials Provided:

1. Anti- SP2/0 Coated Microtiter Plate (96 wells) – 1 no
2. SP2/0 HCP Standards, (0.5 ml/vial) – 0, 2, 10, 50, 100, 200, 300 ng/ml
3. Anti- SP2/0:HRP Conjugate – 6ml
4. Wash Buffer (20X) – 25ml
5. Sample Diluent – 12ml
6. TMB Substrate – 12ml
7. Stop Solution – 12ml
8. Instruction Manual

Materials to be provided by the End-User:

1. Microtiter Plate Reader able to measure absorbance at 450 nm and 630 nm
2. Adjustable pipettes and multichannel pipettor to measure volumes ranging from 25µl to 1000µl
3. Distilled water
4. Wash bottle or automated microplate washer
5. Semi-log paper or software for data analysis
6. Timer
7. Absorbent paper

Handling/Storage:

1. All reagents should be stored at 2ºC to 8ºC.
2. All the reagents and wash solutions are stable until the expiration date of the kit.
3. Prior to use, bring all components to room temperature (18-25 ºC). Store all the components of the kit at its appropriate storage condition.
4. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Health Hazard Warnings:

1. All the reagents provided may be harmful if ingested, inhaled or absorbed through the skin.
2. For Research or Manufacturing use only.

Reagent Preparation (all reagents should be diluted immediately prior to use):

1. Bring all reagents to Room Temperature prior to use.
2. To make Wash Buffer (1X), add 5ml of Wash Buffer (20X) in 95ml of DI water.

Procedural Notes:

1. For good assay reproducibility and sensitivity, proper washing of the ELISA plate to remove excess/unbound reagents is essential.
2. If the HCP concentration of the undiluted sample is less than the diluted sample, this may be indicative of the Hook Effect. High Dose Hook Effect may be observed in samples with very high concentrations of HCP, usually in samples from the initials stages of purifications. To overcome Hook Effect samples to be assayed should be sufficiently diluted with our recommended diluent.
3. Avoid assay of Samples containing sodium azide (NaN3), as it could destroy the HRP activity of the conjugate resulting in under-estimation of the SP2/0 Host Cell Proteins.
4. All Standards and Samples should be assayed at least in duplicates.
5. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
6. If the Substrate has a distinct blue color prior to use it may have been contaminated and use of such substrate can lead to compromisation of the sensitivity of the assay.
7. The plates should be read within 30 minutes after adding the Stop Solution.
8. Make a work list in order to identify the location of Standards and Samples.

Assay Procedure:

***Note:** ALL STEPS MUST BE PERFORMED AT 37°C*

1. Bring all reagents to room temperature prior to use. It is strongly recommended that all Standards and Samples be run in duplicates. A standard curve is required for each assay.
2. Pipette out 50µl of **Anti- SP2/0:HRP Conjugate** into each well.
3. Pipette out 100µl of **Standards** and **Samples** into the respective wells as mentioned in the work list.
4. Cover the plate and incubate it for 90 minutes at 37°C.
5. Aspirate and wash plate 5 times with **Wash Buffer (1X)** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe off any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.
6. Add 100µl of **TMB Substrate** solution and incubate in the dark for 30 minutes at 37°C. Monitor the color development at every 10 minutes
7. Pipette out 100µl of **Stop Solution**. Wells should turn from blue to yellow in color.
8. Read the absorbance at 450 nm blanking on the zero standard.

Example of a Work list

Well #	Contents	Abs at 450nm	Mean Absorbance	ng/ml HCP equiv.
1A 2A	Zero Std Zero Std			
1B 2B	2ng/ml 2ng/ml			
1C 2C	10ng/ml 10ng/ml			
1D 2D	50ng/ml 50ng/ml			
1E 2E	100ng/ml 100ng/ml			
1F 2F	200ng/ml 200ng/ml			
1G 2G	300ng/ml 300ng/ml			
1H 2H	Sample A Sample A			

Calculation of Results:

It is recommended to use the data reduction program (logit-log) in the reader to determine the HCP concentration in the unknown samples. Plot the % bound on the vertical axis (logit) against the HCP concentrations on the horizontal axis (log) for each standard (except the zero standard).

Alternatively, calculate percent conjugate bound (%B) for each standard, and sample relative to the maximum binding (B_0, Zero standard) wells as follows:

$$\%B/B_0 = \frac{\text{Mean Absorbance (highest standard) - Mean Absorbance (standard/sample)}}{\text{Mean Absorbance (highest standard) - Mean Absorbance (Zero standard)}} \times 100$$

Using semi-log graph, plot % bound on the vertical axis against HCP concentration on the horizontal axis for each of the calibrators and draw a smooth line curve through the points. HCP concentrations for the unknown may then be estimated from the line by interpolation.

Of the methods surveyed the logistic-log and fully specified logit-log functions are the most accurate models for forming standard curves and for interpolating HCP concentrations from the standard curve. The accuracy of the fully specified logit-log function is highly dependent on the precise specification of two unknown quantities, the optical densities at zero and infinite concentrations, prior to fitting the model to a typical set of calibration data. The function does not require pre-specification of any parameters before estimating the standard curve, and the four parameters are readily interpretable in terms of identifiable physical quantities. This model also has the advantage that it is easiest to visualize since it does not incorporate complex transformations of the optical density scale.

Precautions:

Do not mix reagents from different kits or lots. Reagents and/or antibodies from different manufacturers should not be used with this set.

Quality Control:

It is recommended that for each laboratory assay appropriate quality control samples in each run to be used to ensure that all reagents and procedures are correct.

Safety Precautions:

• **This kit is for in vitro use only**. Follow the working instructions carefully.

• The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reconstituted reagents.

• Do not use or mix reagents from different lots.

• Do not use reagents from other manufacturers.

▪ Avoid time shift during pipetting of reagents.

• All reagents should be kept at 2 - 8 °C before use in the original shipping container.

• Some of the reagents contain small amounts (< 0.1 % w/w) sodium azide as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.

• Source materials maybe derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.

• Since the kit contains potentially hazardous materials, the following precautions should be observed

- Do not smoke, eat or drink while handling kit material

- Always use protective gloves
- Never pipette material by mouth
- Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.

• In any case GLP should be applied with all general and individual regulations to the use of this kit.

LIMITED WARRANTY

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