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User's Manual

MRC5 HCP ELISA kit

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

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

This kit is intended for use in determining the presence of MRC5 host cell protein (HCP) impurities in products manufactured by recombinant expression in MRC5 host cells. The kit is for Research and Manufacturing Use Only and is not intended for diagnostic use in humans or animals.

General Description

Recombinant expression by the MRC5 cell line is a relatively simple and cost-effective method for production of viral vectors for gene therapies and viral vaccines. These are intended for use as therapeutic agents in humans and animals and as such must be highly purified. The manufacturing and purification process of these products leaves the potential for impurities by HCPs from lysed MRC5 cells. Such impurities can reduce the efficacy of the therapeutic agent and result in adverse toxic or immunological reactions and thus it is desirable to reduce HCP impurities to the lowest levels practical. This simple-to-use,

objective, and semi-quantitative ELISA is a sensitive and specific method to aid in optimal purification process development, process control, and in routine quality control and product release testing.

This kit is "generic" in the sense that it is intended to react with essentially all of the HCPs that could contaminate the product independent of the purification process. The antibodies have been generated against and affinity purified using MRC5 HCPs recovered from lysed MRC5 cells. The antibodies used in this kit were characterized by Antibody Affinity Extraction (AAE) and Mass Spectrometry, demonstrating reactivity to the majority of HCPs.

Special procedures were utilized in the generation of these antibodies to ensure that low molecular weight and less immunogenic impurities as well as high molecular weight components would be represented. As such, this kit can be used as a process development tool to monitor the optimal removal of host cell impurities as well as in routine final product release.

This highly sensitive ELISA kit was qualified for testing of final product HCPs by using actual in-process and final drug substance samples. Each user of this kit is encouraged to perform a similar qualification study to demonstrate it meets their analytical needs. The suitability of this kit for a given sample type and product must be determined and qualified experimentally by each laboratory.

Principles of Testing

The MRC5 assay is a two-site immuno-enzymatic assay. Samples containing MRC5 HCPs are reacted simultaneously with a horseradish peroxidase (HRP) enzyme labeled anti-MRC5 in microtiter strips coated with an affinity purified capture anti-MRC5 antibody. The immunological reactions result in the formation of a sandwich complex of solid phase antibody-HCP-enzyme labeled antibody. The microtiter strips are washed to remove any unbound reactants. The substrate, tetramethylbenzidine (TMB), is then reacted. The amount of hydrolyzed substrate is read on a microtiter plate reader and is directly proportional to the concentration of MRC5 HCPs present.

Reagents And Materials Provided

1. Anti-MRC5 2G:HRP Conjugate

Affinity purified goat antibody conjugated to HRP in a protein matrix with preservative.1×12mL

2. Anti-MRC5 2G Coated Microtiter Strips

12× 8 well strips in a bag with desiccant

3. MRC5 2G HCP Standards

MRC5 HCPs in bovine serum albumin with preservative. Standards at 0, 10, 20, 40, 80, 160, and 320ng/mL.1mL/vial

4. Stop Solution

0. 5M sulfuric acid.1×12mL

5. TMB Substrate

3, ',5,5'Tetramethylbenzidine. 1×12mL

Wash Concentrate (20×)

Tris buffered saline with preservative. 1×50mL

Materials Required But Not Supplied

Microtiter plate reader spectrophotometer with dual wavelength capability at 450 & 650nm. (If your plate reader does not provide dual wavelength analysis you may read at just the 450nm wavelength.)

Pipettors - 50µL and 100µL

Repeating or multichannel pipettor - 100µL

Microtiter plate rotator (400-600 rpm)

Sample Diluent

Distilled water

One(1) liter wash bottle for diluted wash solution

Storage

1. The kit standards mut be removed and stored at -20°C.
2. All other reagents should be stored at 2°C to 8°C for stability until the expiration date printed on the kit.
3. After prolonged storage, you may notice a salt precipitate and/or yellowing of the wash concentrate. These changes will not impact assay performance. To dissolve the precipitate, mix the wash concentrate thoroughly and dilute as directed in the Preparation of Reagents section.

Reagent Preparation

Bring all reagents to room temperature.

Dilute 20× wash concentrate to 1x in 1 liter of distilled water, label with kit lot and expiration date, and store at 4°C.

Assay Procedure

Procedural Notes:

Complete washing of the plates to remove excess unreacted reagents is essential to good assay reproducibility and sensitivity. The manual wash procedure described below generally provides lower backgrounds, higher specific absorbance, and better precision than automated plate washers. If duplicate CVs are poor, or if the absorbance of the '0' standard is greater than 0.300, evaluate plate washing procedure for proper performance.

1. The protocol specifies use of an approved orbital microtiter plate shaker for the immunological steps. These can be purchased from most laboratory supply companies. If you do not have such a device, it is possible to incubate the plate without shaking; however, it will be necessary to extend the immunological incubation step in the plate by about one hour in order to achieve comparable results to the shaking protocol. Do not shake during the 30-minute substrate incubation step, as this may result in higher backgrounds and worse precision.
2. Bring all reagents to room temperature.
3. Set-up plate spectrophotometer to read dual wavelength at 450nm for the test wavelength and ~650nm for the reference.
4. Thorough washing is essential to proper performance of this assay. The manual method described in the assay protocol is preferred for best precision, sensitivity, and accuracy. A more detailed discussion of this procedure can be obtained from our Technical Services Department or on our web site.
5. All standards, controls, and samples should be assayed at least in duplicate.
6. Maintain a repetitive timing sequence from well to well for all assay steps to ensure that all incubation times are the same for each well.
7. Make a work list for each assay to identify the location of each standard, control, and sample.
8. It is recommended that your laboratory assay appropriate quality control samples in each run to ensure that all reagents and procedures are correct. You are strongly urged to make controls in your typical sample matrix using HCPs derived from your cell line. These controls can be aliquoted into single use vials and stored frozen for long-term stability.
9. Strips should be read within 30 minutes after adding stop solution since color will fade over time.

Procedure:

1. Pipette 100µL of anti-MRC5 2G: HRP into each well.
2. Pipette 50µL of standards, controls and samples into wells indicated on work list.
3. Cover & incubate on orbital shaker at 400 - 600rpm for 3 hour at room temperature, 24°C + 4°C.
4. Dump contents of wells into waste. Blot and gently but firmly tap over absorbent paper to remove most of the residual liquid. Overly aggressive banging of the plate to remove all residual liquid is not necessary and may cause variable dissociation of antibody bound material resulting in lower ODs and worse precision. Fill wells generously to overflowing with diluted wash solution using a squirt bottle or by pipetting in ~350µL. Dump and tap again. Repeat for a total of 4 washes. Wipe off any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. Do not allow wash solution to remain in wells for longer than a few seconds. Do not allow wells to dry before adding substrate.
5. Pipette 100µL of TMB substrate.
6. Incubate at room temperature for 30 minutes. DO NOT SHAKE.

7. Pipette 100µL of Stop Solution.
8. Read absorbance at 450/650nm.

Calculation

The standards may be used to construct a standard curve with values reported in ng/mL "total immuno-reactive HCP equivalents". This data reduction may be performed through computer methods using curve-fitting routines such as point-to-point, cubic spline, or 4 parameters logistic fit. Do not use linear regression analysis to interpolate values for samples as this may lead to significant inaccuracies! Data may also be manually reduced by plotting the absorbance values of the standard on the y-axis versus concentration on the x-axis and drawing a smooth point-to-point line. Absorbances of samples are then interpolated from this standard curve.

Quality Control

Precision on duplicate samples should yield average % coefficients of variation of less than 10% for samples in the range of 20-320ng/mL. CVs for samples less than 20ng/mL may be greater than 10%.

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

Typical Standard Curve

Well #	Contents	Abs. at 450-650nm	Mean Abs.
G1	Zero Std	0.168	0.169
G2	Zero Std	0.169	
F1	10ng/mL	0.261	0.269
F2	10ng/mL	0.277	
E1	20ng/mL	0.365	0.374
E2	20ng/mL	0.384	
D1	40ng/mL	0.547	0.554
D2	40ng/mL	0.560	
C1	80ng/mL	0.944	0.947
C2	80ng/mL	0.950	
B1	160ng/mL	1.669	1.684
B2	160ng/mL	1.700	
A1	320ng/mL	2.812	2.835
A2	320ng/mL	2.858	

Precision

Both intra-assay (n=20 replicates) and inter-assay (n=10 assays) precision were determined on 4 controls with low (~17 ng/mL), low-medium (~30 ng/mL), medium (~125 ng/mL), and high (~250 ng/mL) concentrations. The % CV is the standard deviation divided by the mean and multiplied by 100.

Control	Intra assay CV	Inter assay CV
Low	7.8%	5.6%
Low-medium	6.0%	3.4%
Medium	5.2%	3.1%
High	6.6%	4.3%

Sensitivity

The lower limit of detection (LOD) is defined as that concentration corresponding to a signal three standard deviations above the mean of the zero standard. LOD is ~1.8 ng/mL.

The lower limit of quantitation (LLOQ) is defined as the lowest concentration the assay can accurately measure. This is determined by testing the assay at several low concentration points and then interpolating that concentration which corresponds to a nominal recovery of +/-15% and precision of <20% CV. The LLOQ is ~10 ng/mL.

Specificity

Cross reactivity to non-HCP components has not been extensively investigated with this kit. You should evaluate components in your samples for positive interferences such as cross reactivity and non-specific binding.

Interferences

In-process and final formulation drug substances were evaluated by adding known amounts of MRC5 HCP preparation used to make the standards in this kit. All of these samples yielded acceptable recovery defined as between 80-120%. The standards used in this kit contain 4mg/mL of bovine serum albumin intended to simulate non-specific protein effects of most sample proteins. However, very high concentrations of some products may interfere in the accurate measurement of HCPs. In general, extremes in pH (less than 5.0 and greater than 8.5), high salt concentration, high polysaccharide concentrations, urea, organic solvents, and most detergents can cause under-recovery. Each user should qualify that their sample matrices yield accurate recovery. Such an experiment can be performed by diluting the 320ng/mL standard provided with this kit into the sample matrix in question as described in the "Limitations" section.

Precautions

For Research or Manufacturing use only.

Stop reagent is 0.5M H_2SO_4. Avoid contact with eyes, skin, and clothing.

This kit should only be used by qualified technicians.

Limitations

1. Before relying exclusively on this assay to detect host cell proteins, each laboratory should qualify that the

kit antibodies and assay procedure yield acceptable specificity, accuracy, and precision.

2. The standards used in this assay are comprised of MRC5 HCPs solubilized by mechanical disruption and detergent. AAE and Mass Spectrometry analysis of the antibodies used in this kit demonstrates that they recognize the HCPs in the standards preparation.
3. Certain sample matrices may interfere in this assay. The standards used in this kit attempt to simulate typical sample protein and matrices. However, the potential exists that the product itself or other components in the sample matrix may result in either positive or negative interference in this assay. High or low pH, detergents, urea, high salt concentrations, and organic solvents are some of the known interference factors. It is advised to test all sample matrices for interference by diluting the 320ng/mL standard 1 part to 4 parts of the matrix containing no or very low HCP impurities. This diluted standard, when assayed as an unknown, should give an added HCP value in the range of 52 to 76ng/mL.
4. Avoid the assay of samples containing sodium-azide (NaN_3) which will destroy the HRP activity of the conjugate and could result in the under-estimation of HCP levels.
5. If a sample diluent is required, verify its suitability by performing a spike-in test. Compare the absorbance of the sample dilution to that of the Zero Standard to see if there are any discrepancies. Perform spike additions at medium, high, and low concentrations to see if the recovery meets quality control standards. Alternatively, contact Creative Diagnostics to purchase their sample diluent.