CaptureSelect™ G-CSF Select Ligand Leakage ELISA

Catalog Number 810313001 and 810313010

Pub. No. 4486473 **Rev.** B

Item	Description	Storage conditions
Coating reagent (green label)	Goat IgG anti-G-CSF Select affinity ligand, 100 µL	-20°C (-4°F)
Standard solution (blue label)	CaptureSelect™ G-CSF affinity ligand, 100 µL	
Biotinylated reagent (yellow label)	Biotinylated Goat IgG anti-G-CSF Select affinity ligand, 100 µL	

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **www.lifetechnologies.com/support**.

Product description

The CaptureSelect™ G-CSF Select Ligand Leakage ELISA (Enzyme Linked Immuno-Sorbent Assay) is designed for the detection of 1 ng/mL G-CSF affinity ligand that may be present in product purified with GE-Healthcare's G-CSF Select affinity media, which contains the G-CSF affinity ligand as capturing agent.. The assay is designed to minimize interference and to provide accurate quantitation in the presence of human granulocyte colony-stimulating factor and other proteins. The G-CSF Select Ligand Leakage ELISA can be used as a tool to aid in optimal purification process development and in routine quality control of in-process streams as well as final product.

Principle of the assay

The CaptureSelect™ ligand leakage assay enables detection of the affinity ligand in solutions with and without the presence of the target protein. These sandwich assays involve the following steps:

- A microtiter plate is coated with affinity-purified anti-affinity ligand polyclonal goat antibodies.
- Samples containing the affinity ligand are incubated in the coated plate wells.
- Bound affinity ligand is detected by biotinylated affinity-purified anti-affinity ligand polyclonal goat antibodies.
- Streptavidin horseradish peroxidase conjugate is added to bind to the biotinylated antibody in the sandwich complex.
- Substrate reactive with horseradish peroxidase (tetramethylbenzidine-hydrogen peroxide) is added.
- The amount of hydrolyzed substrate is determined and is directly proportional to the concentration of affinity ligand present.

Required materials and equipment (not provided)

- PBS: Phosphate buffered saline pH 7.4
- PBST: Phosphate buffered saline (PBS) pH 7.4 + 0.05 (v/v)% Tween® 20 Solution
- Bovine Serum Albumin (BSA), Fraction V 99% pure (Sigma-Aldrich A3059)

  Note: Other qualities of Bovine Serum Albumin or other blocking proteins might result in higher background levels.
- Dilution Buffer A: 0.05 (v/v)% Tween® 20 Solution in PBS pH 7.4
- 2X Dilution Buffer A: 0.1 (v/v)% Tween® 20 Solution in PBS pH 7.4
- Blocking solution: 4 (w/v)% BSA in PBS pH 7.4
- Streptavidin-Horseradish Peroxidase diluted immediately before using according to manufacturer guidelines
- Tetramethylbenzidine (TMB) and hydrogen peroxide (H_2O_2) substrate (prepare 1:1 solution immediately before use)
- 1 M H_2SO_4
- Microtiter plate (Maxisorp, Nunc)
- Microtiter plate shaker
- Microtiter plate reader (450 nm)
- Milli-Q® water

Procedure

Coat the plate

1. Make a 1:100 dilution of the Coating reagent with PBS pH 7.4.
2. Add 100 µL diluted Coating reagent to each well in a microtiter plate and incubate overnight at 4°C (39°F).

Prepare standards

1. Prepare a 6.4 µg/mL stock Standard solution: Add 10 µL Standard solution to 770 µL Dilution Buffer A.
2. Using the stock Standard solution from step step 1, prepare a standard dilution series according to the table below.

Tube	Concentration (ng/mL)	Standard	Dilution Buffer A
1	64.0	10 µL diluted Standard solution	990 µL
2	16.0	250 µL 64.0 ng/mL	750 µL
3	8.0	500 µL 16.0 ng/mL	500 µL
4	4.0	500 µL 8.0 ng/mL	500 µL
5	2.0	500 µL 4.0 ng/mL	500 µL
6	1.0	500 µL 2.0 ng/mL	500 µL
7	0.5	500 µL 1.0 ng/mL	500 µL
8	0.25	500 µL 0.5 ng/mL	500 µL
9	0	0	500 µL

Prepare assay samples

Dilute 75 µL sample with 75 µL of 2X Dilution Buffer A.

ELISA assay procedure

1. Block the plate:
   a. Wash the coated plate 5 times with PBST.

b. Add 200 µL/well of Blocking solution to the coated plate. Leave at room temperature for 30 minutes on a microtiter plate shaker.
c. Wash the plate 1 time with PBST.

2. Add samples and standards:
   a. Add 100 µL of each concentration of the standard dilution series (0 to 64.0 ng/mL) or sample to appropriate wells.
   b. Incubate the plate 1 hour at room temperature on a microtiter plate shaker.
   c. Wash the plate 5 times with PBST.
3. Add Biotinylated reagents:
   a. Make a 1:100 dilution of the Biotinylated reagents with Dilution Buffer A.
   b. Add 100 µL diluted Biotinylated reagents to each well and incubate the plate 1 hour at room temperature.
   c. Wash the plate 5 times with PBST.
4. Add diluted Streptavidin-Horseradish peroxidase:
   a. Dilute in Dilution Buffer A according to the manufacturer's guidelines.
   b. Add 100 µL diluted Streptavidin-Horseradish peroxidase to each well containing sample or standard.
   c. Incubate the plate 1 hour at room temperature on a microtiter plate shaker.
   d. Wash the plate 5 times with PBST.
   e. Wash the plate 2 times with Milli-Q® water.
5. Develop and read the plate:
   a. Add 100 µL 1:1 mixed TMB/H_2O_2 substrate per well.
   b. Incubate the plate for approximately 20 minutes on a microtiter plate shaker.
   c. When the background signal starts to develop, add 50 µL 1 M H_2SO_4 to stop the coloring reaction and achieve a maximal signal-to-noise ratio.
   d. Measure the OD of the microtiter plate at 450 nm with a microtiter plate reader.

Calculate results

Construct a standard curve with values reported in ng/mL. Use curve-fitting routines such as 4-parameter logistic fit. Do not use linear regression analysis to interpolate values for samples, which may lead to significant inaccuracies.

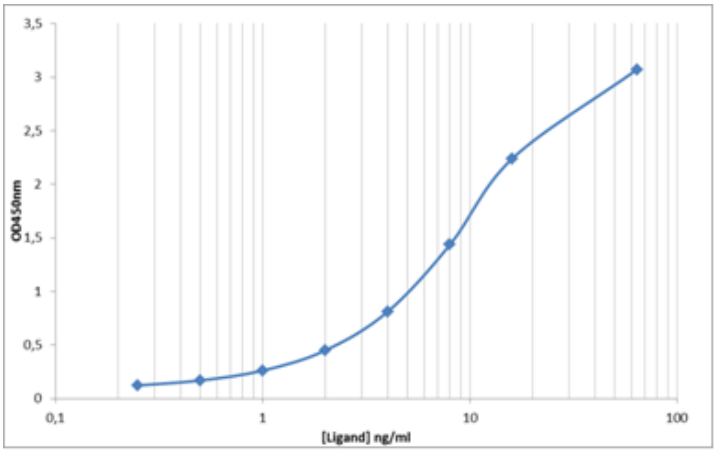

Figure 1 Example calibration curve G-CSF Select ligand leakage assay

Validate the assay

Perform validation studies that include at least the following experiments to validate this kit for your application: 1) Intra- and inter-assay precision experiments to establish reproducibility, 2) Recovery experiments using test samples with known amounts of the 500 µg/mL Standard solution, which is included in the kit.

Ordering information

CaptureSelect™ G-CSF Select Ligand Leakage ELISA	Part number
1 assay	810313001
10 assays	810313010

For more information

For more information on CaptureSelect™ products and ligand leakage ELISA products, go to **www.lifetechnologies.com/captureselect**.

Obtaining SDSs

Safety Data Sheets (SDSs) are available from **www.lifetechnologies.com/support**.

Note: For SDSs of chemicals from third-party manufacturers, contact the chemical manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

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