



ELISA Kit

Catalog # **KHC2031 (96 tests)**
 KHC2032 (192 tests)

Human **G-CSF**

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Table of Contents

Table of Contents.....	3
Contents and Storage.....	4
Introduction.....	5
Purpose.....	5
Principle of the Method.....	5
Background Information.....	5
Methods.....	6
Materials Needed But Not Provided.....	6
Procedural Notes.....	6
Preparation of Reagents.....	7
Assay Procedure.....	8
Typical Data.....	9
Performance Characteristics.....	10
Sensitivity.....	10
Precision.....	10
Linearity of Dilution.....	10
Recovery.....	11
Specificity.....	11
High Dose Hook Effect.....	11
Expected Values.....	11
Limitations of the Procedure.....	11
Appendix.....	12
Troubleshooting Guide.....	12
Technical Support.....	13
Citation.....	14

Contents and Storage

Storage

Store at 2 to 8°C.

Contents

Reagents Provided	96 Test Kit	192 Test Kit
<i>Hu G-CSF Standard</i> , recombinant Hu G-CSF. Contains 0.1% sodium azide. Refer to vial label for quantity and reconstitution volume.	2 vials	4 vials
<i>Standard Diluent Buffer</i> . Contains 0.1% sodium azide. Diluent contains human plasma which was screened negative for HIV 1/2, HTLV I/II and hepatitis B and C; 25 mL per bottle.	1 bottle	2 bottles
<i>Antibody Coated Wells. 12 x 8 Well Strips.</i>	1 plate	2 plates
<i>Hu G-CSF Biotin Conjugate</i> (Biotin-labeled anti-G-CSF). Contains 0.1% sodium azide; 6 mL per bottle.	1 bottle	2 bottles
<i>Streptavidin-HRP (100X)</i> . Contains 3.3 mM thymol; 0.125 mL per vial.	1 vial	2 vials
<i>Streptavidin-HRP Diluent</i> . Contains 3.3 mM thymol; 25 mL per bottle.	1 bottle	1 bottle
<i>Wash Buffer Concentrate (25X)</i> . 100 mL per bottle.	1 bottle	1 bottle
<i>Stabilized Chromogen, Tetramethylbenzidine (TMB)</i> . 25 mL per bottle.	1 bottle	1 bottle
<i>Stop Solution</i> . 25 mL per bottle.	1 bottle	1 bottle
<i>Plate Covers, adhesive strips.</i>	3	4

Disposal Note

This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state and local regulations for disposal.

Safety

All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of infectious agents.

Introduction

Purpose

The Invitrogen Human Granulocyte Colony Stimulating Factor (Hu G-CSF) ELISA is to be used for the *in vitro* quantitative determination of Hu G-CSF in human serum, plasma, buffered solution, or cell culture medium. This assay will recognize both natural and recombinant Hu G-CSF.

For Research Use Only. CAUTION: Not for human or animal therapeutic or diagnostic use.

Principle of the Method

The Invitrogen Hu G-CSF kit is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA). A monoclonal antibody specific for Hu G-CSF has been coated onto the wells of the microtiter strips provided. Samples, including standards of known Hu G-CSF content, control specimens, and unknowns, are pipetted into these wells, followed by the addition of a biotinylated monoclonal second antibody.

During the first incubation, the Hu G-CSF antigen binds simultaneously to the immobilized (capture) antibody on one site, and to the solution phase biotinylated antibody on a second site.

After removal of excess second antibody, Streptavidin-Peroxidase (enzyme) is added. This binds to the biotinylated antibody to complete the four-member sandwich. After a second incubation and washing to remove all the unbound enzyme, a substrate solution is added, which is acted upon by the bound enzyme to produce color. The intensity of this colored product is directly proportional to the concentration of Hu G-CSF present in the original specimen.

Background Information

Colony-stimulating factors (CSFs) are glycoprotein molecules that support growth of hematopoietic colonies. These factors were isolated and characterized following the observation that colonies containing mature neutrophils and macrophages develop when immobilized hematopoietic cells are conditioned with various media. The major clinically available CSFs are granulocyte macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF), macrophage colony-stimulating factor (M-CSF) and interleukin-3 (IL-3). G-CSF regulates the proliferation, differentiation and survival of cells in the granulocytic lineage. G-CSF is secreted as a glycoprotein monomer containing 174 amino acids and two intramolecular disulphide bonds, both of which are necessary for proper folding and biological activity. X-ray diffraction analysis reveals a four α -helical bundle structure. G-CSF exerts its activity by binding to a receptor that is a type I membrane protein of the hematopoietic growth factor receptor family. This family of receptors lacks intrinsic catalytic activity. G-CSF binding causes the receptor monomers to dimerize. The dimeric receptor then interacts with and activates one or more of the JAK protein tyrosine kinases. Epitope mapping experiments using neutralizing antibodies indicate that the amino acid residues from 20 to 46, and the carboxy terminus are important for binding to the receptor.

Methods

Materials Needed But Not Provided

- Microtiter plate reader (at or near 450 nm) with software
 - Calibrated adjustable precision pipettes
 - Distilled or deionized water
 - Plate washer: automated or manual (squirt bottle, manifold dispenser, etc.)
 - Glass or plastic tubes for diluting solutions
 - Absorbent paper towels
 - Calibrated beakers and graduated cylinders
 - 37°C Incubator
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Procedural Notes

1. When not in use, kit components should be refrigerated. All reagents should be warmed to room temperature before use.
 2. Microtiter **plates should be allowed to come to room temperature before opening the foil bags.** Once the desired number of strips has been removed, immediately reseal the bag and store at 2 to 8°C to maintain plate integrity.
 3. Samples should be collected in pyrogen/endotoxin-free tubes.
 4. Samples should be frozen if not analyzed shortly after collection. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well prior to analysis.
 5. When possible, avoid use of badly hemolyzed or lipemic sera. If large amounts of particulate matter are present, centrifuge or filter prior to analysis.
 6. It is recommended that all standards, controls and samples be run in duplicate.
 7. When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation times for all wells.
 8. **Do not mix or interchange different reagent lots from various kit lots.**
 9. Do not use reagents after the kit expiration date.
 10. Absorbances should be read immediately, but can be read up to 2 hours after assay completion. For best results, keep plate covered in the dark.
 11. In-house controls should be run with every assay. If control values fall outside pre-established ranges, the accuracy of the assay is suspect.
 12. All residual wash 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.
 13. Because Stabilized *Chromogen* is light sensitive, avoid prolonged exposure to light. Avoid contact between chromogen and metal, or color may develop.
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Directions for Washing

- **Incomplete washing will adversely affect the test outcome.** All washing must be performed with the *Wash Buffer Concentrate (25X)* provided.
 - Washing can be performed manually as follows: completely aspirate the liquid from all wells by gently lowering an aspiration tip into the bottom of each well. Take care not to scratch the inside of the well. After aspiration, fill the wells with at least 0.4 ml of diluted *Wash Buffer*. Let soak for 15 to 30 seconds, then aspirate the liquid. Repeat as directed under Assay Procedure. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.
 - Alternatively, the diluted *Wash Buffer* may be put into a squirt bottle. If a squirt bottle is used, flood the plate with the diluted *Wash Buffer*, completely filling all wells. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.
 - If using an automated washer, follow the washing instructions carefully.
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Preparation of Reagents

Dilution of Standard

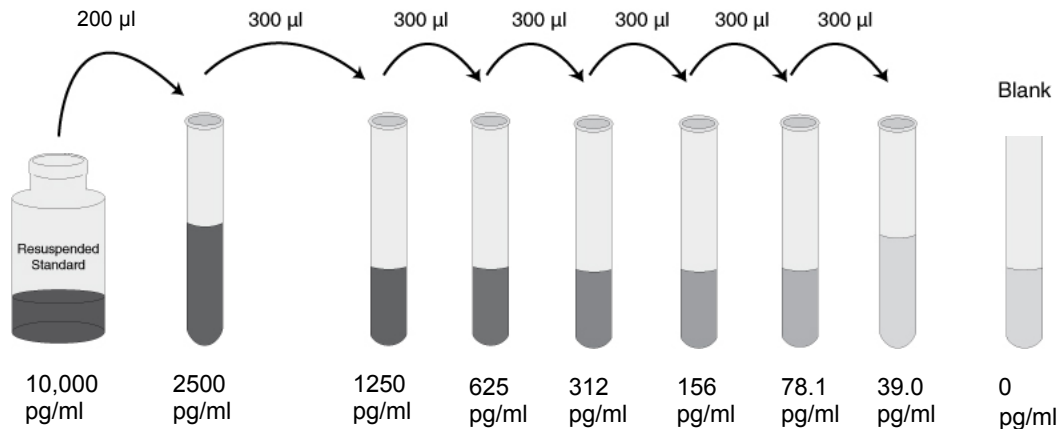
This assay has been calibrated against the WHO reference preparation 88/502 (version 4). One nanogram equals 600 International Units.

Note: Either glass or plastic tubes may be used for standard dilutions.

1. Reconstitute standard to 10,000 pg/ml with *Standard Diluent Buffer*. Refer to standard vial label for instructions. Swirl or mix gently and allow to sit for 10 minutes to ensure complete reconstitution. Use standard within 1 hour of reconstitution.
2. Add 0.200 ml of the reconstituted standard to a tube containing 0.600 ml *Standard Diluent Buffer*. Label as 2500 pg/ml Hu G-CSF. Mix.
3. Add 0.300 ml of *Standard Diluent Buffer* to each of 6 tubes labeled 1250, 625, 312, 156, 78.1, and 39.0 pg/ml Hu G-CSF.
4. Make serial dilutions of the standard as described in the following dilution diagram. Mix thoroughly between steps.

Note

Discard all remaining reconstituted and diluted standards after completing assay. Return the *Standard Diluent Buffer* to the refrigerator.



Preparing SAV-HRP

Note: Prepare within 15 minutes of usage, as activity decreases. The *Streptavidin-HRP (100X)* is in 50% glycerol. This solution is viscous. To ensure accurate dilution, allow *Streptavidin-HRP (100X)* to reach room temperature. Gently mix. Pipette *Streptavidin-HRP (100X)* slowly. Remove excess concentrate solution from pipette tip by gently wiping with clean absorbent paper.

1. Dilute 10 µL of this 100x concentrated solution with 1 ml of *Streptavidin-HRP Diluent* for each 8-well strip used in the assay. Label as Streptavidin-HRP Working Solution.
2. Return the unused *Streptavidin-HRP (100X)* to the refrigerator.

# of 8-Well Strips	Volume of Streptavidin-HRP (100X)	Volume of Diluent
2	20 µL solution	2 ml
4	40 µL solution	4 ml
6	60 µL solution	6 ml
8	80 µL solution	8 ml
10	100 µL solution	10 ml
12	120 µL solution	12 ml

Dilution of Wash Buffer

1. Allow the *Wash Buffer Concentrate (25X)* to reach room temperature and mix to ensure that any precipitated salts have redissolved. Dilute 1 volume of the *Wash Buffer Concentrate (25X)* with 24 volumes of deionized water (e.g., 50 ml may be diluted up to 1.25 liters, 100 ml may be diluted up to 2.5 liters). Label as Working Wash Buffer.
 2. Store both the concentrate and the Working Wash Buffer in the refrigerator. The diluted buffer should be used within 14 days.
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Assay Procedure

Be sure to read the *Procedural Notes* section before carrying out the assay.

Allow all reagents to reach room temperature before use. Gently mix all liquid reagents prior to use.

Note: A standard curve must be run with each assay.

1. Determine the number of 8-well strips needed for the assay. Insert these in the frame(s) for current use. (Re-bag extra strips and frame. Store these in the refrigerator for future use.)
2. Add 100 μ L of the *Standard Diluent Buffer* to the zero standard wells. Well(s) reserved for chromogen blank should be left empty.
3. Add 100 μ L of the standards to the appropriate microtiter wells. For all samples (serum, plasma, buffered solution, cell culture, and controls), add 50 μ L of *Standard Diluent Buffer* to each well followed by 50 μ L of sample. Tap gently on side of plate to mix. See **Preparation of Reagents**.
4. Pipette 50 μ L of biotinylated *Hu G-CSF Biotin Conjugate* solution into each well except the chromogen blank(s). Tap gently on the side of the plate to mix.
5. Cover plate with *plate cover* and incubate for **2 hours at 37°C**.
6. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **Directions for Washing**.
7. Add 100 μ L Streptavidin-HRP Working Solution to each well except the chromogen blank(s). See **Preparation of Reagents**.
8. Cover plate with the *plate cover* and incubate for **30 minutes at room temperature**.
9. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **Directions for Washing**.
10. Add 100 μ L of *Stabilized Chromogen* to each well. The liquid in the wells will begin to turn blue.
11. Incubate for **30 minutes at room temperature and in the dark**. **Note: Do not cover the plate with aluminum foil or metalized mylar.** The incubation time for chromogen substrate is often determined by the microtiter plate reader used. Many plate readers have the capacity to record a maximum optical density (O.D.) of 2.0. The O.D. values should be monitored and the substrate reaction stopped before the O.D. of the positive wells exceeds the limits of the instrument. The O.D. values at 450 nm can only be read after the *Stop Solution* has been added to each well. If using a reader that records only to 2.0 O.D., stopping the assay after 20 to 25 minutes is suggested.
12. Add 100 μ L of *Stop Solution* to each well. Tap side of plate gently to mix. The solution in the wells should change from blue to yellow.
13. Read the absorbance of each well at 450 nm having blanked the plate reader against a chromogen blank composed of 100 μ L each of *Stabilized Chromogen* and *Stop Solution*. Read the plate within 2 hours after adding the *Stop Solution*.

14. Use a curve fitting software to generate the standard curve. A four parameter algorithm provides the best standard curve fit.
 15. Read the Hu G-CSF concentrations for unknown samples and controls from the standard curve. **Multiply value(s) obtained for sample(s) by 2 to correct for the 1:2 dilution in step 3.** (Samples producing signals greater than that of the highest standard should be further diluted in *Standard Diluent Buffer* and reanalyzed, multiplying the concentration found by the appropriate dilution factor.)
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**Typical
Data
(Example)**

The following data were obtained for the various standards over the range of 0 to 2500 pg/ml Hu G-CSF.

Standard Hu G-CSF (pg/ml)	Optical Density (450 nm)
2500	2.7735
1250	1.627
625	0.964
312	0.556
156	0.341
78.1	0.2345
39.0	0.191
0	0.1575

Performance Characteristics

Sensitivity The minimum detectable dose of Hu G-CSF is < 20 pg/ml. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 30 times.

Precision 1. Intra-Assay Precision
 Samples of known Hu G-CSF concentration were assayed in replicates of 12 to determine precision within an assay.

	Sample 1	Sample 2	Sample 3
Mean (pg/ml)	250	591	757
SD	12.9	41.0	53.3
%CV	5.2	6.9	7.0
SD = Standard Deviation CV = Coefficient of Variation			

2. Inter-Assay Precision
 Samples were assayed 36 times in multiple assays to determine precision between assays.

	Sample 1	Sample 2	Sample 3
Mean (pg/ml)	273	547	778
SD	22.6	49.9	66.9
%CV	8.3	9.1	8.6
SD = Standard Deviation CV = Coefficient of Variation			

Linearity of Dilution Human serum and tissue culture medium containing 10% fetal calf serum were spiked with Hu G-CSF and serially diluted in *Standard Diluent Buffer* over the range of the assay. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient of 0.99 in both cases.

Dilution	Serum			Cell Culture		
	Measured (pg/ml)	Expected (pg/ml)	% Expected	Measured (pg/ml)	Expected (pg/ml)	% Expected
neat	2148	-	-	1942	-	-
1/2	911	1074	85	996	971	103
1/4	460	537	86	479	486	99
1/8	310	269	115	223	243	92
1/16	124	134	93	122	121	101

Recovery The recovery of Hu G-CSF added to human serum averaged 83% (70% to 92%, N = 8). The recovery of Hu G-CSF added to tissue culture medium containing 1% fetal bovine serum averaged 108%, while the recovery of Hu G-CSF added to tissue culture medium containing 10% fetal bovine serum averaged 113%.

High Dose Hook Effect Samples spiked with Hu G-CSF up to 1.5 mg/mL gave responses higher than that obtained for the last standard point.

Expected Values Twenty serum and twenty plasma (EDTA) samples were evaluated in this assay. All samples measured <39 pg/mL (the lowest Hu G-CSF standard).

Specificity Buffered solutions of a panel of substances at 10,000 pg/ml were assayed with the Invitrogen Hu G-CSF kit. The following substances were tested and found to have no cross-reactivity: human IL-1 β , IL-2, sIL-2R, IL-3, IL-4, IL-5, sIL-6R, IL-7, IL-8, IL-10, IL-12, IL-13, IL-15, IL-16, GM-CSF, IFN- γ , TNF- α ; mouse IL-3, G-CSF, GM- CSF.

Limitations of the Procedure Do not extrapolate the standard curve beyond the top standard point; the dose-response is non-linear in this region and accuracy is difficult to obtain. Dilute all samples above the top standard point with *Standard Diluent Buffer*, reanalyze these and multiply results by the appropriate dilution factor.

The influence of various drugs, aberrant sera (hemolyzed, hyperlipidemic, jaundiced, etc.) and the use of biological fluids in place of serum samples have not been thoroughly investigated. The rate of degradation of native Hu G-CSF in various matrices has not been investigated. The immunoassay literature contains frequent references to aberrant signals seen with some sera, attributed to heterophilic antibodies. Though such samples have not been seen to date, the possibility of this occurrence cannot be excluded.

Appendix

Troubleshooting Guide

Elevated background

Cause: Insufficient washing and/or draining of wells after washing. Solution containing either biotin or Streptavidin-HRP can elevate the background if residual is left in the well.

Solution: Wash according to the protocol. Verify the function of automated plate washer. At the end of each washing step, invert plate on absorbent tissue on countertop and allow to completely drain, tapping forcefully if necessary to remove residual fluid.

Cause: Contamination of substrate solution with metal ions or oxidizing reagents.

Solution: Use distilled/deionized water for dilution of wash buffer and use plastic equipment. DO NOT COVER plate with foil.

Cause: Contamination of pipette, dispensing reservoir or substrate solution with SAV-HRP conjugate.

Solution: Do not use chromogen that appears blue prior to dispensing onto the plate. Obtain new vial of chromogen.

Cause: Incubation time is too long or incubation temperature is too high.

Solution: Reduce incubation time and/or temperature.

Elevated sample/standard ODs

Cause: Incorrect dilution of standard stock solution; intermediary dilutions not followed correctly.

Solution: Follow the protocol instructions regarding the dilution of the standard.

Cause: Incorrect dilution of the Streptavidin-HRP Working Solution.

Solution: Warm solution of Streptavidin-HRP (100X) to room temperature, draw up slowly and wipe tip with kim-wipe to remove excess. Dilute ONLY in Streptavidin-HRP Diluent provided.

Cause: Incubation times extended.

Solution: Follow incubation times outlined in protocol.

Cause: Incubations carried out at 37°C when RT is dictated.

Solution: Perform incubations at RT (= 25 ± 2°C) when instructed in the protocol.

Poor standard curve

Cause: Improper preparation of standard stock solution.

Solution: Dilute lyophilized standard as directed by the vial label only with the standard diluent buffer or in a diluent that most closely matches the matrix of your sample.

Cause: Reagents (lyophilized standard, standard diluent buffer, etc.) from different kits, either different cytokine or different lot number, were substituted.

Solution: NEVER substitute any components from another kit.

Cause: Errors in pipetting the standard or subsequent steps.

Solution: Always dispense into wells quickly and in the same order. Do not touch the pipette tip on the individual microwells when dispensing. Use calibrated pipettes and the appropriate tips for that device.

Weak/no
color
develops

Cause: Reagents not at RT ($25 \pm 2^\circ\text{C}$) at start of assay.

Solution: Allow ALL reagents to warm to RT prior to commencing assay.

Cause: Incorrect storage of components, e.g., not stored at 2 to 8°C .

Solution: Store all components exactly as directed in protocol and on labels.

Cause: Working Streptavidin-HRP solution made up longer than 15 minutes before use in assay.

Solution: Use the diluted Streptavidin-HRP within 15 minutes of dilution.

Cause: TMB solution lost activity.

Solution 1: The TMB solution should be clear before it is dispensed into the wells of the microtiter plate. An intense aqua blue color indicates that the product is contaminated. Please contact Technical Support if this problem is noted. To avoid contamination, we recommend that the quantity required for an assay be dispensed into a disposable trough for pipetting. Any TMB solution left in the trough should be discarded.

Solution 2: Avoid contact of the TMB solution with items containing metal ions.

Cause: Attempt to measure analyte in a matrix for which the ELISA assay has not been optimized.

Solution: Please contact Technical Support for advice when using nonvalidated sample types.

Cause: Wells have been scratched with pipette tip or washing tips.

Solution: Use caution when dispensing and aspirating into and out of microwells.

Poor
Precision

Cause: Errors in pipetting the standards, samples or subsequent steps.

Solution: Always dispense into wells quickly and in the same order. Do not touch the pipette tip on the individual microwells when dispensing. Use calibrated pipettes and the appropriate tips for that device. Check for any leaks in the pipette tip.

Cause: Repetitive use of tips for several samples or different reagents.

Solution: Use fresh tips for each sample or reagent transfer.

Cause: Wells have been scratched with pipette tip or washing tips.

Solution: Use caution when dispensing and aspirating into and out of microwells.

Technical Support

Contact Us

For more troubleshooting tips, information, or assistance, please call, email, or go online to www.invitrogen.com/ELISA.



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Citations

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For a up-to-date and complete list, visit www.invitrogen.com/ELISA or contact Technical Support.














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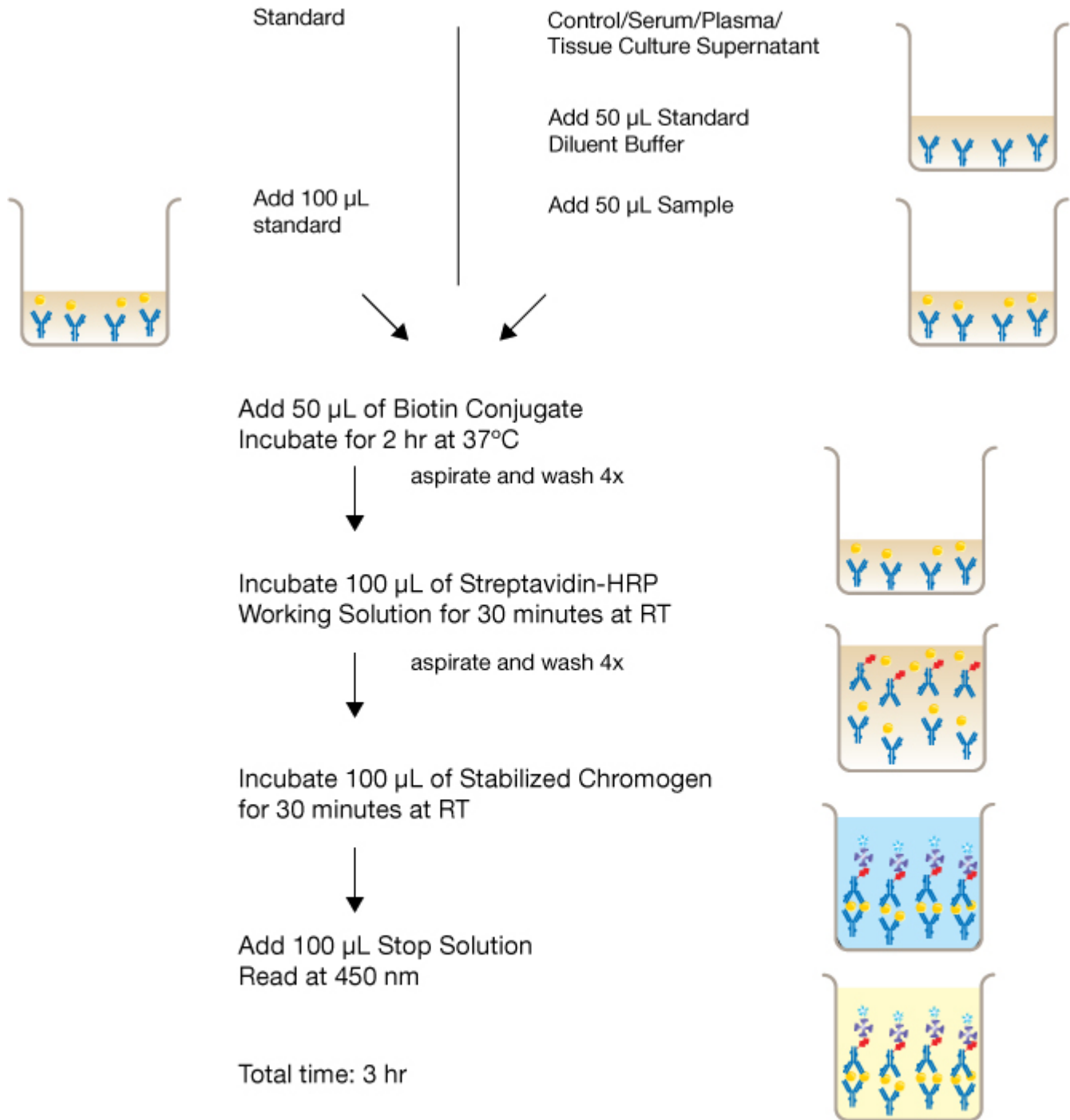
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Explanation of symbols

Symbol	Description	Symbol	Description
	Catalogue Number		Batch code
	Research Use Only		<i>In vitro</i> diagnostic medical device
	Use by		Temperature limitation
	Manufacturer		European Community authorised representative
	Without, does not contain		With, contains
	Protect from light		Consult accompanying documents
	Directs the user to consult instructions for use (IFU), accompanying the product.		

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Human G-CSF Assay Summary



 G-CSF	 Streptavidin-HRP	 Anti-G-CSF	 Biotinylated Anti-G-CSF
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