

# **Magnetic Luminex<sup>®</sup> Performance Assay**

## **Human Base Kit A**

Catalog Number LUHM000

For the simultaneous quantitative determination of multiple human cytokine concentrations in cell culture supernates, serum, and plasma.

**This package insert must be read in its entirety before using this product.  
For research use only. Not for use in diagnostic procedures.**

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## INTRODUCTION

Cytokines are intercellular signaling proteins released from a wide variety of cells and tissues. They play an integral role in regulating growth and cellular proliferation as well as modulating host response to infection, injury, and inflammation. Cytokines also influence reproduction and bone remodeling. A large number of cytokines are pleiotropic and share similar functions. In addition, many cytokines influence the production of other cytokines. Analysis and quantification of cytokines in biological fluids and cell culture supernates has thus become increasingly important. Methods such as bioassay, enzyme-linked immunosorbent assay (ELISA), intracellular staining, ribonuclease protection assay (RPA) and polymerase chain reaction (PCR) have all been used for quantifying cytokines; however, each of these techniques has limitations associated with it. These techniques are not capable of measuring multiple cytokines simultaneously in a limited sample volume.

Any combination of the following bead sets are suitable for use with this base kit.

Analyte	Catalog Number	Microparticle Region
CXCL5/ENA-78	LUHM254	12
FGF basic	LUHM233	13
G-CSF	LUHM214	14
GM-CSF	LUHM215	15
IFN- $\gamma$	LUHM285	18
IL-1 $\alpha$ /IL-1F1	LUHM200	19
IL-1 $\beta$ /IL-1F2	LUHM201	20
IL-1 $\alpha$ /IL-1F3	LUHM280	21
IL-2	LUHM202	22
IL-4	LUHM204	25
IL-5	LUHM205	26
IL-6	LUHM206	27
CXCL8/IL-8	LUHM208	28
IL-10	LUHM217	29
IL-17	LUHM317	30
CCL2/MCP-1	LUHM279	33
CCL3/MIP-1 $\alpha$	LUHM270	34
CCL4/MIP-1 $\beta$	LUHM271	35
CCL5/RANTES	LUHM278	36
TNF- $\alpha$	LUHM210	37
Thrombopoietin	LUHM288	38
VEGF	LUHM293	39

## PRINCIPLE OF THE ASSAY

Magnetic Luminex® Performance Assay multiplex kits are designed for use with the Luminex® MAGPIX® CCD Imager. Alternatively, kits can be used with the Luminex® 100/200™, Luminex® FLEXMAP 3D®, or Bio-Rad® Bio-Plex®, dual laser, flow-based sorting and detection platforms.

Analyte-specific antibodies are pre-coated onto magnetic microparticles embedded with fluorophores at set ratios for each unique microparticle region. Microparticles, standards and samples are pipetted into wells and the immobilized antibodies bind the analytes of interest. After washing away any unbound substances, a biotinylated antibody cocktail specific to the analytes of interest is added to each well. Following a wash to remove any unbound biotinylated antibody, streptavidin-phycoerythrin conjugate (Streptavidin-PE), which binds to the biotinylated antibody, is added to each well. Final washes remove unbound Streptavidin-PE, the microparticles are resuspended in buffer and read using the Luminex® MAGPIX® Analyzer. A magnet in the analyzer captures and holds the superparamagnetic microparticles in a monolayer. Two spectrally distinct Light Emitting Diodes (LEDs) illuminate the microparticles. One LED excites the dyes inside each microparticle to identify the region and the second LED excites the PE to measure the amount of analyte bound to the microparticle. A sample from each well is imaged with a CCD camera with a set of filter to differentiate excitation levels.

Analysis with the Luminex® 100/200™, Luminex® FLEXMAP 3D®, or Bio-Rad Bio-Plex uses one laser to excite the dyes inside each microparticle to identifying the microparticle region and the second laser to excite the PE to measure the amount of analyte bound to the microparticle. All excitation emitted as each microparticle passes through the flow cell is then analyzed to differentiate excitation levels using a Photomultiplier Tube (PMT) and an Avalanche Photodiode.

## LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples fall outside the dynamic range of the assay, further dilute the samples with the appropriate calibrator diluent and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until these proteins have been tested in the Luminex® Performance Assay, the possibility of interference cannot be excluded.
- Magnetic Luminex® Performance Assays afford the user the benefit of multi-analyte analysis of biomarkers in a single complex sample. For each sample type, a single multipurpose diluent is used to optimize recovery, linearity, and reproducibility. Such a multipurpose diluent may not optimize any single analyte to the same degree that a unique diluent selected for analysis of that analyte can optimize conditions. Therefore, some performance characteristics may be more variable than those for assays designed specifically for single analyte analysis.
- **Only the analytes listed on the Standard Value Card can be measured with this base kit.**

## MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED, DILUTED, OR RECONSTITUTED MATERIAL
Standard Cocktail 1	895531	2 vials of recombinant human cytokines in a buffered protein base with preservatives; lyophilized.	Discard after use. Use fresh standards for each assay.
Standard Cocktail 2	895546	2 vials of recombinant human cytokines in a buffered protein base with preservatives; lyophilized.	
Microparticle Diluent	895529	6 mL of a buffered protein base with blue dye and preservative.	May be stored for up to 1 month at 2-8 °C.* <i>Once diluted, 1X solutions must be discarded. Use fresh diluents for each assay.</i>
Biotin Antibody Diluent 2	895832	5.5 mL of a buffered protein base with preservative.	
Streptavidin-PE	892525	0.07 mL of a concentrated streptavidin-phycoerythrin conjugate with preservatives.	
Calibrator Diluent RD5K	895119	21 mL of a 2-fold concentrated solution of a buffered protein base with preservatives. <i>For cell culture supernate samples. Use diluted 1:2 in this assay.</i>	May be stored for up to 1 month at 2-8 °C.*
Calibrator Diluent RD6-40	895817	21 mL of a buffered animal serum with preservatives. <i>For serum/plasma samples. May contain a precipitate. Mix well before and during use.</i>	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. <i>May turn yellow over time.</i>	
Microplate	641385	1 flat-bottomed 96-well microplate used as a vessel for the assay.	
Mixing Bottles	895505	2 empty 8 mL bottles used for mixing microparticles with Microparticle Diluent.	
Plate Sealers	640445	4 adhesive foil strips.	
Standard Value Card 1	750215	1 card listing the Standard Cocktail 1 reconstitution volume and working standard concentrations for this lot of base kit.	
Standard Value Card 2	750618	1 card listing the Standard Cocktail 2 reconstitution volume and working standard concentrations for this lot of base kit.	

\*Provided this is within the expiration date of the kit.

## OTHER SUPPLIES REQUIRED

- **Magnetic Luminex® Performance Assay analyte-specific kit(s) (see page 1).**
- Luminex® MAGPIX®, Luminex® 100/200™, Luminex® FLEXMAP 3D®, or Bio-Rad Bio-Plex analyzer with X-Y platform.
- Hand-held microplate magnet or platewasher with a magnetic platform.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Multi-channel pipette, manifold dispenser, or automated dispensing unit.
- 50 mL and 500 mL graduated cylinders.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of  $800 \pm 50$  rpm.
- Microcentrifuge.
- **Polypropylene** test tubes for dilution of standards and samples.
- Luminex® Performance Assay Controls (optional; R&D Systems®, Catalog # QC02).

## PRECAUTIONS

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the SDS on our website prior to use.

## TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Protect microparticles and Streptavidin-PE from light at all times to prevent photobleaching.

## SAMPLE COLLECTION & STORAGE

**The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.**

**Cell Culture Supernates** - Remove particulates by centrifugation and assay immediately or aliquot and store samples at  $\leq -20$  °C. Avoid repeated freeze-thaw cycles.

**Serum** - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifuging for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at  $\leq -20$  °C. Avoid repeated freeze-thaw cycles.

**Plasma** - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at  $\leq -20$  °C. Avoid repeated freeze-thaw cycles.

## SAMPLE PREPARATION

**Use polypropylene tubes.**

**Note:** *On the day of the assay, ALL fresh and previously frozen serum and plasma samples require centrifugation at 16,000 x g for 4 minutes immediately prior to use or dilution.*

Serum and plasma samples require a 4-fold dilution. A suggested 4-fold dilution is 30  $\mu$ L of sample + 90  $\mu$ L of Calibrator Diluent RD6-40. Mix thoroughly.

When assaying CCL5/RANTES, serum and plasma samples must be further diluted 25-fold to a final 100-fold dilution. A suggested 100-fold dilution is 10  $\mu$ L of the 4-fold diluted sample + 240  $\mu$ L of Calibrator Diluent RD6-40. Mix thoroughly.

## REAGENT PREPARATION

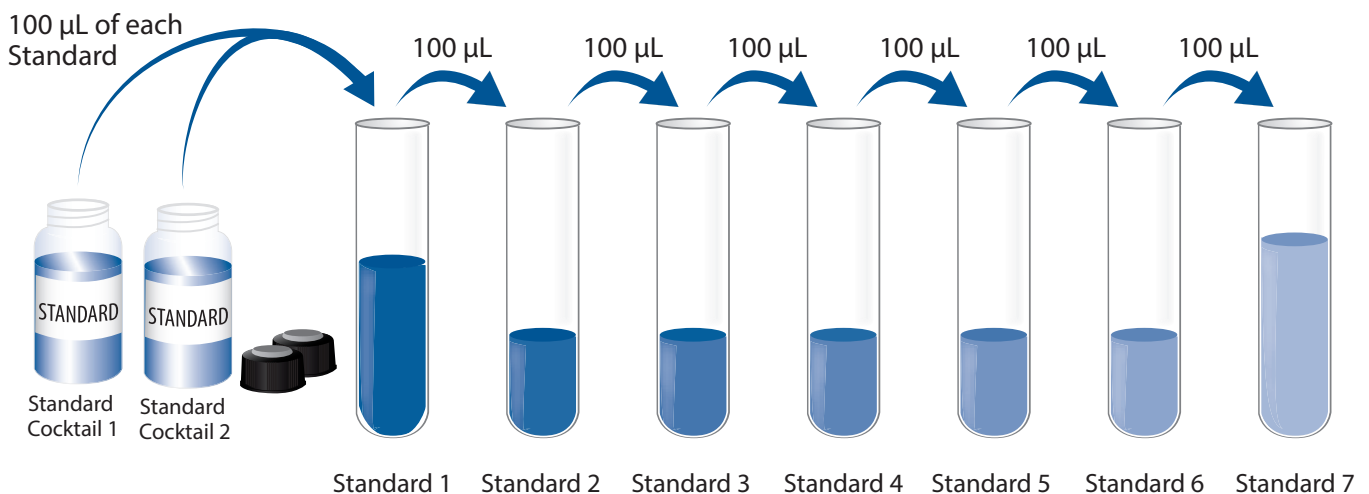
**Bring all reagents to room temperature before use.**

**Wash Buffer** - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

**Calibrator Diluent RD5K (diluted 1:2)** - **For cell culture supernate samples only.** Add 20 mL of Calibrator Diluent RD5K to 20 mL of deionized or distilled water to prepare 40 mL of Calibrator Diluent RD5K (diluted 1:2).

**Standards** - **Refer to the Standard Value Cards for the reconstitution volumes and assigned values.** Reconstitute 1 each of Standard Cocktails 1 and 2 with Calibrator Diluent RD5K (diluted 1:2) (*for cell culture supernate samples*) or Calibrator Diluent RD6-40 (*for serum/plasma samples*). Calibrator Diluent RD6-40 may contain a precipitate. Mix well before and during use. Allow the standards to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. This produces a 5X stock of each Standard Cocktail.

**Use polypropylene tubes.** Pipette 300  $\mu\text{L}$  of the appropriate calibrator diluent into a tube labeled working standard 1. Pipette 200  $\mu\text{L}$  of the appropriate calibrator diluent into the remaining tubes. Pipette 100  $\mu\text{L}$  of each of the 5X reconstituted Standard Cocktail vials 1 and 2 into the working standard 1 tube. Use working standard 1 to produce a 3-fold dilution series (below). Refer to the analyte specific datasheet for details. Mix each tube thoroughly before the next transfer. Working standard 1 serves as the high standard. The appropriate calibrator diluent serves as the blank.





## DILUTED MICROPARTICLE COCKTAIL PREPARATION

1. Centrifuge each Microparticle Concentrate vial for 30 seconds at 1000 x g prior to removing the cap.
2. Gently vortex the vials to resuspend the microparticles, taking precautions not to invert the vials.
3. Dilute the Microparticle Concentrates in the mixing bottle provided. The volume of the Microparticle Concentrate listed in the table below is for each analyte (e.g. if measuring a full plate of IL-1 $\beta$  and IL-6, add 50  $\mu$ L of IL-1 $\beta$  Microparticle Concentrate and 50  $\mu$ L of IL-6 Microparticle Concentrate to 5 mL of Microparticle Diluent).

Number of Wells Used	Microparticle Concentrate	+	Microparticle Diluent
96	50.0 $\mu$ L	+	5.00 mL
72	37.5 $\mu$ L	+	3.75 mL
48	25.0 $\mu$ L	+	2.50 mL
24	12.5 $\mu$ L	+	1.25 mL

**Note:** Protect microparticles from light during handling. Diluted microparticles cannot be stored. Prepare microparticles within 30 minutes of use.

## DILUTED BIOTIN-ANTIBODY COCKTAIL PREPARATION

1. Centrifuge each Biotin-Antibody Concentrate vial for 30 seconds at 1000 x g prior to removing the cap.
2. Gently vortex the vials, taking precautions not to invert the vials.
3. Dilute the Biotin-Antibody Concentrates in Biotin Antibody Diluent 2. The volume of the Biotin-Antibody listed in the table below is for each analyte (e.g. if measuring a full plate, add 50  $\mu$ L of each Biotin-Antibody to 5 mL of Biotin Antibody Diluent 2). Mix gently.

Number of Wells Used	Biotin-Antibody Concentrate	+	Biotin Antibody Diluent
96	50.0 $\mu$ L	+	5.00 mL
72	37.5 $\mu$ L	+	3.75 mL
48	25.0 $\mu$ L	+	2.50 mL
24	12.5 $\mu$ L	+	1.25 mL

## STREPTAVIDIN-PE PREPARATION

**Use a polypropylene amber bottle or a polypropylene tube wrapped with aluminum foil. Protect Streptavidin-PE from light during handling and storage.**

1. Centrifuge the Streptavidin-PE vial for 30 seconds at 1000 x g prior to removing the cap.
2. Gently vortex the vial, taking precautions not to invert the vial.
3. Dilute the Streptavidin-PE concentrate in Wash Buffer.

Number of Wells Used	Streptavidin-PE Concentrate	+	Wash Buffer
96	55.0 $\mu$ L	+	5.50 mL
72	42.0 $\mu$ L	+	4.10 mL
48	28.0 $\mu$ L	+	2.75 mL
24	14.0 $\mu$ L	+	1.35 mL

## INSTRUMENT SETTINGS

**Note:** *Adjust the probe height setting on the analyzer to avoid puncturing the plate. Calibrate the analyzer using the proper reagents for superparamagnetic microparticles (refer to instrument manual).*

### **Luminex® MAGPIX® analyzer:**

- a) Sample volume: 50 µL
- b) Assign the microparticle region for each analyte being measured (see page 1)
- c) 50 count/region
- d) Collect Median Fluorescence Intensity (MFI)

### **Luminex® 100/200™, Luminex® FLEXMAP 3D® and Bio-Rad Bio-Plex analyzers:**

**Note:** *Ensure that the instrument flow rate is set to the default of 60 µL/minute (fast) for all flow based analyzers.*

- a) Sample volume: 50 µL
- b) Bead Type:
  - i. Luminex® 100/200™ and FLEXMAP 3D® select MagPlex
  - ii. Bio-Rad Bio-Plex Manager use Bio-Plex MagPlex Beads (Magnetic)
- c) Doublet Discriminator gates:
  - i. Luminex® 100/200™ and FLEXMAP 3D® set at 8000 and 16,500
  - ii. Bio-Rad Bio-Plex Manager set at 8000 and 23,000
- d) Reporter Gain Setting:
  - i. Luminex® 100/200™ use Default setting
  - ii. Luminex® FLEXMAP 3D® use Standard PMT setting
  - iii. Bio-Rad Bio-Plex Manager use the low RP1 target value for the CAL2 setting
- e) Assign the microparticle region for each analyte being measured (see page 1)
- f) 50 count/region
- g) Collect MFI

## ASSAY PROCEDURE

**Bring all reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples be assayed in duplicate.**

**Note:** *Protect microparticles and Streptavidin-PE from light at all times.*

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Add 50  $\mu\text{L}$  of standard, control, or sample\* per well. A plate layout is provided to record standards and samples assayed.
3. Resuspend the diluted Microparticle Cocktail by inversion or vortexing. Add 50  $\mu\text{L}$  of the microparticle cocktail to each well of the microplate. Securely cover with a foil plate sealer. Incubate for 3 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at  $800 \pm 50$  rpm.
4. Using a magnetic device designed to accommodate a microplate, wash by applying the magnet to the bottom of the microplate, allow 1 minute before removing the liquid, filling each well with Wash Buffer (100  $\mu\text{L}$ ) and allow 1 minute before removing the liquid again. Complete removal of liquid is essential for good performance. **Note: Do NOT blot; this may cause a loss of microparticles.** Perform the wash procedure three times.

**Note:** *Refer to the magnetic device user manual for proper wash technique using a round bottom microplate.*

5. Add 50  $\mu\text{L}$  of diluted Biotin-Antibody Cocktail to each well. Securely cover with a foil plate sealer and incubate for 1 hour at room temperature on the shaker set at  $800 \pm 50$  rpm.
6. Repeat the wash as in step 4.
7. Add 50  $\mu\text{L}$  of diluted Streptavidin-PE to each well. Securely cover with a foil plate sealer and incubate for 30 minutes at room temperature on the shaker set at  $800 \pm 50$  rpm.
8. Repeat the wash as in step 4.
9. Resuspend the microparticles by adding 100  $\mu\text{L}$  of Wash Buffer to each well. Incubate for 2 minutes on the shaker set at  $800 \pm 50$  rpm.
10. Read within 90 minutes using a Luminex® or Bio-Rad analyzer.  
**Note:** *Resuspend microparticles immediately prior to reading by shaking the plate for 2 minutes on the plate shaker at  $800 \pm 50$  rpm.*

\*Samples may require dilution. See Sample Preparation section.

## ASSAY PROCEDURE SUMMARY

**Note:** Protect microparticles and Streptavidin-PE from light at all times.

- 1 Prepare all reagents as instructed.
- 2 Add 50  $\mu$ L of standard, control, or sample\* to each well.
- 3 Add 50  $\mu$ L of diluted Microparticle Cocktail to each well.  
Incubate for 3 hours at RT on a shaker at 800 rpm.
- 4 Wash by removing the liquid from each well, filling with 100  $\mu$ L Wash Buffer, and removing the liquid again.  
Perform the wash 3 times.
- 5 Add 50  $\mu$ L of diluted Biotin-Antibody Cocktail to each well.  
Cover and incubate for 1 hour at RT on the shaker at 800 rpm.
- 6 Repeat the wash as in step 4.
- 7 Add 50  $\mu$ L of diluted Streptavidin-PE to each well.  
Incubate for 30 minutes at RT on the shaker at 800 rpm.
- 8 Repeat the wash as in step 4.
- 9 Add 100  $\mu$ L of Wash Buffer to each well.  
Incubate for 2 minutes at RT on the shaker at 800 rpm.
- 10 Read within 90 minutes using a Luminex<sup>®</sup> or Bio-Rad analyzer  
**Note:** Resuspend microparticles immediately prior to reading.

\*Samples may require dilution. See Sample Preparation section.

## **CALCULATION OF RESULTS**

Use the Standard concentrations on the Standard Value Card and calculate 3-fold dilutions for the remaining levels. Average the duplicate readings for each standard and sample and subtract the average blank Median Fluorescence Intensity (MFI).

Create a standard curve for each analyte by reducing the data using computer software capable of generating a five parameter logistic (5-PL) curve-fit.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

## **CALIBRATION**

This assay is calibrated against highly purified recombinant human cytokines produced at R&D Systems®.

## SPECIFICITY

The assay was tested for cross-reactivity and interference with the following factors. Less than 0.5% cross-reactivity and interference was observed unless otherwise noted on the analyte specific datasheet.

### Recombinant human:

6Ckine	IL-12 p40
CNTF	IL-12 p70
β-ECGF	IL-13
FGF acidic	IL-15
FGF-4	IL-16
FGF-5	IL-17
FGF-6	IL-18
FGF-9	LIF
FGF-10	LIF R
FGF-18	MIP-1α
GCP-2	MIP-3α
GROα	MIP-3β
GROβ	MCP-2
GROγ	MCP-3
I-309	MCP-4
IGF-I	M-CSF
IGF-II	TNF RI
IL-1 RI	VEGF <sub>121</sub>
IL-1 RII	VEGF <sub>165</sub>
IL-2 Rα	VEGF-D
IL-2 Rβ	
IL-2 Rγ	
IL-3 Rα	
IL-4 R	
IL-5 Rα	
IL-6 R	
IL-10 R	
IL-3	
IL-7	
IL-9	
IL-11	

### Recombinant mouse:

G-CSF
GM-CSF
IFN-γ
IL-1α
IL-1ra
IL-2
IL-4
IL-5
IL-6
IL-8
IL-10
IL-17
MIP-1α
MIP-1β
RANTES
Tpo
TNF-α
VEGF

### Recombinant rat:

GM-CSF
IFN-γ
IL-1α
IL-1β
IL-2
IL-4
IL-6
IL-10
TNF-α

### Recombinant porcine:

GM-CSF
IL-1α
IL-1β
IL-2
IL-4
IL-6
IL-8
IL-10
Leptin
TNF-α

### Recombinant human multiplex partners:

ENA-78
FGF basic
GM-CSF
IFN-γ
IL-1α
IL-1β
IL-1ra
IL-2
IL-4
IL-5
IL-6
IL-8
IL-10
IL-17
MCP-1
MIP-1α
MIP-1β
RANTES
Tpo
TNF-α
VEGF

## PLATE LAYOUT

Use this plate layout to record standards and samples assayed.

12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								
	A	B	C	D	E	F	G	H

**NOTES**

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