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ImmunoGuide®

Instructions for Use

Antibody to Golimumab ELISA

Enzyme immunoassay for the determination of free antibodies to Golimumab in serum and plasma

REF: TM09020

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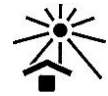

2-8°C

For Research Use Only - Not for Use in Diagnostic Procedures

Manufactured for
Immuno-Biological Laboratories Inc. (IBL-America)
8201 Central Avenue NE,
Suite P Minneapolis,
MN 55432 USA
Tel: 763-780-2955 Toll Free: 1-888-523-1246
www.ibl-america.com

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1. INTENDED USE

Enzyme immunoassay for the determination of free antibodies to Golimumab in serum and plasma.

2. SUMMARY AND EXPLANATION

The drug Golimumab (trade name Simponi®) is a human monoclonal antibody that binds to both the soluble and transmembrane bioactive forms of human TNF-α. This interaction prevents the binding of TNF-α to its receptors, thereby inhibiting the biological activity of TNF.
Golimumab has been proven effective in the treatment of Rheumatoid Arthritis (RA), Ankylosing Spondylitis (AS), Psoriatic Arthritis (PsA) or Ulcerative Colitis (UC). Antibodies to Golimumab were detected in 57 (4%) of Golimumab -treated patients across the Phase 3 RA, PsA, and AS trials through Week 24. The data from the literature demonstrated that Anti-Drug Antibody positivity was significantly associated with low Golimumab levels and poor therapeutic response. The positive correlation between serum drug trough levels and therapeutic response indicates that drug monitoring could be useful for optimising the dosing of biologics in a personalised therapy strategy. The *ImmunoGuide* Antibody to Golimumab ELISA Kit can be efficiently used for monitoring Anti-Golimumab antibodies during therapy and offers the clinician a tool for decision on possible preventive measures to reduce Anti-Golimumab antibodies.

3. PRINCIPLE OF THE TEST

This *ImmunoGuide* anti-drug antibody(ies) (ADA) kit is a bridging type ELISA for the determination of free antibodies against the drug Golimumab in serum and plasma samples. During the first incubation period, ADA in serum or plasma samples are captured by the drug coated on the microtiter wells. After washing away the unbound components from samples, a peroxidase-labelled drug conjugate is added and then incubated. ADA, if present in sample, will make a bridge, with its identical Fab arms, between the drug coated on the well and the other drug molecule labelled with peroxidase. After a second washing step, the bound enzymatic activity is detected by addition of tetramethylbenzidine (TMB) chromogen-substrate. Finally, the reaction is terminated with stop solution. The positive reaction is expected to be related to the presence of ADA in the sample.

4. WARNINGS AND PRECAUTIONS

1. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood. For further information (clinical background, test performance, automation protocols, alternative applications, literature, etc.) please refer to the local distributor.

2. In case of severe damage of the kit package, please contact ***IBL-America*** or your supplier in writing, latest one week after receiving the kit. Do not use damaged components in test runs but keep safe for complaint related issues.
3. Obey lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.
4. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.
5. Reagents of this kit containing hazardous material may cause eye and skin irritations. See MATERIALS SUPPLIED and labels for details.
6. Chemicals and prepared or used reagents have to be treated as hazardous waste according the national biohazard safety guidelines or regulations.
7. Avoid contact with Stop solution. It may cause skin irritations and burns.
8. If any component of this kit contains human serum or plasma it is indicated and if so, it has been tested and were found to be negative for HIV I/II, HBsAg and HCV. However, the presence of these or other infectious agents cannot be excluded absolutely and therefore reagents should be treated as potential biohazards in use and for disposal.
9. Some reagents contain preservatives. In case of contact with eyes or skin, flush immediately with water.

5. STORAGE AND STABILITY OF THE KIT

The kit is shipped at ambient temperature and should be stored at 2-8°C. Keep away from heat or direct sun light. The storage and stability of specimen and prepared reagents is stated in the corresponding chapters. The microtiter strips are stable up to the expiry date of the kit in the broken, but tightly closed bag when stored at 2–8°C.

6. SPECIMEN COLLECTION, HANDLING AND STORAGE

Serum, Plasma (EDTA, Heparin)*

The usual precautions for venipuncture should be observed. It is important to preserve the chemical integrity of a blood specimen from the moment it is collected until it is assayed. Do not use grossly hemolytic, icteric or grossly lipemic specimens. Samples appearing turbid should be centrifuged before testing to remove any particulate material.

Storage:	2-8°C	≤-20°C (Aliquots)	Keep away from heat or direct sun light Avoid repeated freeze-thaw cycles
Stability:	3 d	6 mon	

***** Drug administration/infusion may camouflage/mask the presence of anti-drug antibodies (ADA) in serum/plasma samples. Therefore, blood sampling time is also critical for detection of ADA. It is proposed to obtain blood sample just before administration of the drug.

7. CONTENTS OF THE KIT

QUANTITY	COMPONENT
1 x 12 x 8	**Microtiter Plate** Break apart strips pre-coated with the drug Golimumab.
5 x 1 mL	**Anti-Drug Antibody (ADA) Standards** Standard A-B-C-D-E Ready to use. Used for construction of the standard curve. Contains anti-drug antibody, preservative and stabilizer. The quantity of anti-drug is indicated on the vial label. The vials can be reused several times.
1 x 1 mL	**Positive Control.** Green colored. Ready to use. Contains antibody against drug, preservative and stabilizer. The quantity of anti-drug is indicated on the QCC datasheet. The vials can be reused several times.
1 x 2 mL	**Cut-Off Control.** Yellow colored. Ready to use. Contains antibody against drug, preservative and stabilizer. The vials can be reused several times.
1 x 50 mL	**Dilution Buffer** Orange colored. Ready to use. Contains proteins and preservative.
1 x 12 mL	**Assay Buffer** Blue colored. Ready to use. Contains proteins and preservative.
1 x 12 mL	**Enzyme Conjugate** Red colored. Ready to use. Contains horseradish peroxidase(HRP)-conjugated Golimumab, Proclin® and stabilizers.
1 x 12 mL	**TMB Substrate Solution** Ready to use. Contains 3,3',5,5'-Tetramethylbenzidine (TMB).
1 x 12 mL	**Stop Solution** Ready to use. 1 N Hydrochloric acid (HCl).
1 x 50 mL	**Wash Buffer**, Concentrate (20x) Contains buffer, Tween® 20 and Kathon™.
3 x 1	**Adhesive Seal** For sealing microtiter plate during incubation.

8. MATERIALS REQUIRED BUT NOT SUPPLIED

1. Micropipettes (< 3% CV) and tips to deliver 5-1000 µL.
2. Bidistilled or deionised water and calibrated glasswares.
3. Wash bottle, automated or semi-automated microtiter plate washing system.
4. Microtiter plate reader capable of reading absorbance at 450 nm.
5. Absorbent paper towels, standard laboratory glass or plastic vials, and a timer.

9. PROCEDURE NOTES

1. Any improper handling of samples or modification of the test procedure may influence the results. The indicated pipetting volumes, incubation times, temperatures and pre-treatment steps have to be performed strictly according to the instructions. Use calibrated pipettes and devices only.
2. Once the test has been started, all steps should be completed without interruption. Make sure that required reagents, materials and devices are prepared readily at the appropriate time. Allow all reagents and specimens to reach room temperature (20-25 °C) and gently swirl each vial of liquid reagent and sample before use. Mix reagents without foaming.
3. Avoid contamination of reagents, pipettes and wells/tubes. Use new disposable plastic pipette tips for each reagent, standard or specimen. Do not interchange the caps of vials. Always cap not used vials. Do not reuse wells or reagents.
4. Use a pipetting scheme to verify an appropriate plate layout.
5. Incubation time affects results. All wells should be handled in the same order and time sequences. It is recommended to use an 8-channel Micropipettor for pipetting of solutions in all wells.
6. Microplate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or an automatic microplate washing system. Do not allow the wells to dry between incubations. Do not scratch coated wells during rinsing and aspiration. Rinse and fill all reagents with care. While rinsing, check that all wells are filled precisely with Wash Buffer, and that there are no residues in the wells.
7. Humidity affects the coated wells. Do not open the pouch until it reaches room temperature. Unused wells should be returned immediately to the resealed pouch including the desiccant.

10. PRE-TEST SETUP INSTRUCTIONS

10.1. Preparation of Components*

Dilute/ Dissolve	Component		Diluent	Relation	Remarks	Storage	Stability
10 mL	Wash Buffer	up to 200 mL	Distilled Water	1:20	Warm up at 37°C to dissolve crystals. Mix vigorously.	2-8 °C	4 w

***** Prepare Wash Buffer before starting the assay procedure.

10.2. Dilution of Samples*.

Sample	To be diluted	With	Remarks
Serum/ Plasma	1:2	Dilution Buffer	50 µL Serum/Plasma + 50 µL Dilution Buffer

*. Transfer 50 µL of each sample in a reaction tube and add 50 µL of Dilution Buffer and then mix thoroughly.

11. TEST PROCEDURE

11.1. GENERAL REMARKS

11.1.1. Before performing the assay, samples and assay kit should be brought to room temperature (about 30 minutes beforehand) and ensure the homogeneity of the solution.
11.1.2. All Standards/Controls should be run with each series of unknown samples.
11.1.3. Standards/Controls should be subject to the same manipulations and incubation times as the samples being tested.
11.1.4. All steps of the test should be completed without interruption.
11.1.5. Use new disposable plastic pipette tips for each reagent, standard or specimen in order to avoid cross contamination.
11.1.6. The total pipetting time needed for dispensing all samples into the wells should not exceed 5 minutes. If this is difficult to achieve the samples should be pre-dispensed in a separate neutral polypropylene microplate and then transferred into the reaction ELISA plate by a multi channel pipette.

11.2. ASSAY PROCEDURE

1.	Pipette **100 µl** of **Assay Buffer** into each of the wells to be used.
2.	Pipette **50 µL** of **Ready-to-Use Standards, Positive Control, Cut-Off Control and 1:2 Diluted Samples (Dilution of sample is described in section 10.2)** into the respective wells of the microtiter plate. Wells A1: Standard A B1: Standard B C1: Standard C D1: Standard D E1: Standard E F1: Positive Control G1: Cut-Off Control H1: Cut-Off Control A2 and so on: 1:2 Diluted Samples (Serum/Plasma)
3.	Cover the plate with adhesive seal. Shake plate carefully by tapping several times. **Incubate the plate on a bench top for 60 min** at **room temperature (RT, 20-25°C)**.
4.	Remove adhesive seal. Aspirate or decant the incubation solution. Wash the plate **4 X 350 µL** of **Diluted Wash Buffer** per well. Remove excess solution by tapping the inverted plate gently on a paper towel.
5.	Pipette **100 µL** of **Enzyme Conjugate** (HRP-drug) into each well.
6.	Cover plate with adhesive seal. Shake plate carefully. **Incubate 60 min** at RT.
7.	Remove adhesive seal. Aspirate or decant the incubation solution. Wash the plate **4 X 350 µL** of **Diluted Wash Buffer** per well. Remove excess solution by tapping the inverted plate gently on a paper towel.
8.	Pipette **100 µL** of Ready-to-Use **TMB** Substrate Solution into each well.
9.	**Incubate 15 min** at RT. Avoid exposure to direct sunlight**.**
10.	Stop the substrate reaction by adding **100 µL** of **Stop Solution** into each well. Briefly mix contents by gently shaking the plate. Color changes from blue to yellow.
11.	Measure optical density (OD) with a photometer at **450 nm only** within **15 min** after pipetting the Stop Solution.

11. 3. QUALITY CONTROL

The test results are only valid if the test has been performed following the instructions. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable standards/laws. All standards/controls must be found within the acceptable ranges as stated above and/or label. If the criteria are not met, the run is not valid and should be repeated. In case of any deviation, the following technical issues should be reviewed: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, incubation conditions and washing methods.

11. 4. CALCULATION OF RESULTS

11.4.1. QUALITATIVE INTERPRETATION

The qualitative analysis is performed by using the Cut-Off control which is compatible with the "Limit of Detection (LoD)". The Cut-Off is estimated on 120 samples and evaluated according to NCCLS publication EP17-A, Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline and as briefly outlined below.

- LoD = LoB + 1.645 σs.

Where LoB and σS are the value of Limit of Blank and standard deviation of the population of the low sample measurements, respectively.

- LoB = μB + 1.645 σB

Where μB and σB are the mean and standard deviation of the blank measurements, respectively

The results are evaluated by dividing each individual OD result of 1:2 diluted patient's sample by the Cut-Off Value. Cut-Off Value is determined as described below:

Cut-Off Value (The Mean OD450nm of two wells used for Cut-Off Control) = (OD450nm of the first well used for Cut-Off Control+ OD450nm of the second well used for Cut-Off Control) / 2
(i.e. The sum OD450nm is divided by 2)

11.4.1.2. If the "1:2 diluted sample OD450nm" is less (<) than the "Cut-Off Value", the sample is regarded as NEGATIVE for Anti-Drug-Antibody (ADA) specific for the drug in concern.

11.4.1.3. If the "1:2 diluted sample OD450nm" is equal and higher (≥) than the "Cut-Off Value", the sample is regarded as POSITIVE for Anti-Drug-Antibody (ADA) specific for the drug in concern.

And if required, the OD of 1:2 pre-diluted positive samples may be plotted on the standard curve for quantitative analysis.

OD450nm of 1:2 Diluted Patient Sample	Interpretation
≥ Cut-Off Value	POSITIVE
< Cut-Off Value	NEGATIVE

11.4.2 QUANTITATIVE INTERPRETATION

11.4.2.1. For the calculation of the positive sample concentration, polynomial regression is recommended. Standard curve is constructed by plotting the units of the 5 standard points (ng/mL) along the abscissa (X axis) and the corresponding OD450nm values along the ordinate (Y axis).

11.4.2.2. The positive control value should be comprised into the range indicated on the vial label.

11.4.2.3. The Anti-Drug Antibody (ADA) concentration of positive samples can be reported based on the principles indicated below.

11.4.2.4. The ADA concentration of pre-diluted (at the ratio of 1:2) positive samples can be directly read on the standard curve. In order to report the ADA concentration for the corresponding undiluted positive sample, the value must be multiplied by the factor of 2. Example: If a 1:2 pre-diluted positive sample results in an ADA concentration of 15ng/mL on the standard curve, then its undiluted sample value corresponds to 30ng/mL (15ng/mL x 2 = 30ng/mL).

11.4.2.5. The 1:2 diluted positive samples with OD450nm value higher than that of the highest standard supplied could be further diluted, e.g. 1:20, using Dilution Buffer and then retested to obtain more precise result (see below). In such a situation, the number of units should be multiplied by the selected dilution factor.

Dilution of a high positive sample at 1:20:

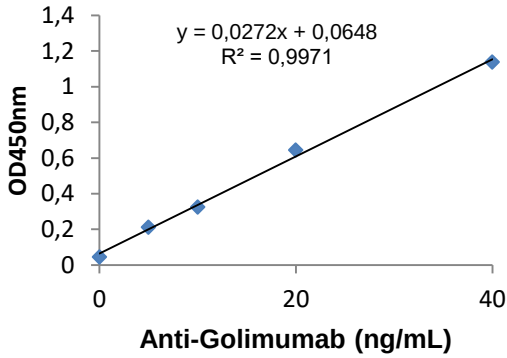
Sample	To be diluted	With	Remarks
Serum/ Plasma	1:20	Dilution Buffer	10 µL Serum/Plasma + 190 µL Dilution Buffer

11.4.2.6. If any diluted positive sample is still reading OD450nm higher than that of highest standard supplied, it should be further diluted (in such a case 1:200 dilution of the sample is proposed) appropriately with Dilution Buffer and then retested. Also this second dilution has to be used for calculation of the final result. It is recommended that each laboratory determines the best dilution ratio for their samples in order to minimize retesting.

11.4.2.7. Rarely, some 1:2 diluted positive samples may produce a value less than the sensitivity level of the assay. In such a situation, it is highly recommended to repeat the test with a new patient's sample obtained at another time but just before the time of drug administration.

Typical Calibration Curve
(Example. Do not use it for calculation!)

Standard	[C] (ng/mL)	Mean OD450nm
A	40	1,138
B	20	0,645
C	10	0,326
D	5	0,213
E	0	0,045



12. ASSAY CHARACTERISTICS

12.1. ANALYTICAL SENSITIVITY-THRESHOLD VALUE

- The Detection Threshold for the assay is 2 ng/mL.
- The assay sensitivity for undiluted clinical samples corresponds to 4 ng/mL Because the serum or plasma samples are instructed to be diluted at two-fold (1:2) before starting the assay.

12.2. ASSAY RANGE

For anti-Golimumab antibodies in serum and plasma, the method has been demonstrated to be highly (>0.99 %) linear from 0 to 40 ng/mL.

12.3. ASSAY PRECISION

Intra-assay CV:

Mean	CV (%)
40	<10
20	<10
10	<10

Inter-assay CV: <10%.

12.4. ASSAY RECOVERY

Recovery rate was found to be 85-115% using native serum and plasma samples spiked with exogenous Anti Drug Antibody (ADA) positive samples.

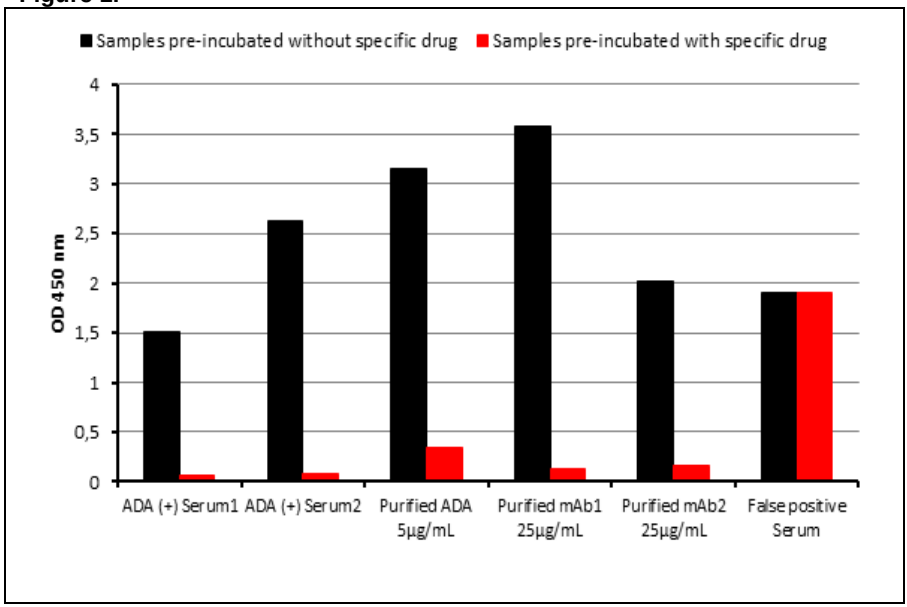
13. AUTOMATION

The *ImmunoGuide* ADA ELISA is suitable also for being used by an automated ELISA processor.

14. SPECIAL NOTES ABOUT ANTI DRUG ANTIBODY (ADA) & ELISA

The detection of ADA formation is highly dependent on the sensitivity and specificity of the assay used. The initial screening assay should be sensitive to low and high-affinity ADA. Endogenous and exogenous components in serum or plasma may influence assay results. Measuring immune responses to therapeutic protein products that possess Ig tails, such as mAb and Fc-fusion proteins, may be particularly difficult when RF is present in serum or plasma.
If the serum/plasma sample of a patient, a candidate for a specific monoclonal drug treatment, is negative at the time before starting drug therapy and become positive during treatment, it could be concluded that the induction of the specific ADA is suspected in this individual. As shown in Figure 1, the true positive reaction of the sample is inhibited by pre-incubation of the sample with the specific drug itself (samples are spiked with certain amount of drug). However, inhibition was not observed in false positive reaction related with other components in sample (Figure 1).
If it is desired to further confirm the specificity of the ADA positive signal, ImmunoGuide suggests a “Confirmatory Assay (competitive drug inhibition test)”: Confirmatory Assay is performed by retesting the positive sample after dilution (at 1:2-1:10) with Dilution Buffer, 0.9% NaCl solution or phosphate buffered saline containing the drug in concern at a concentration of 50µg/mL that is exogenously added.

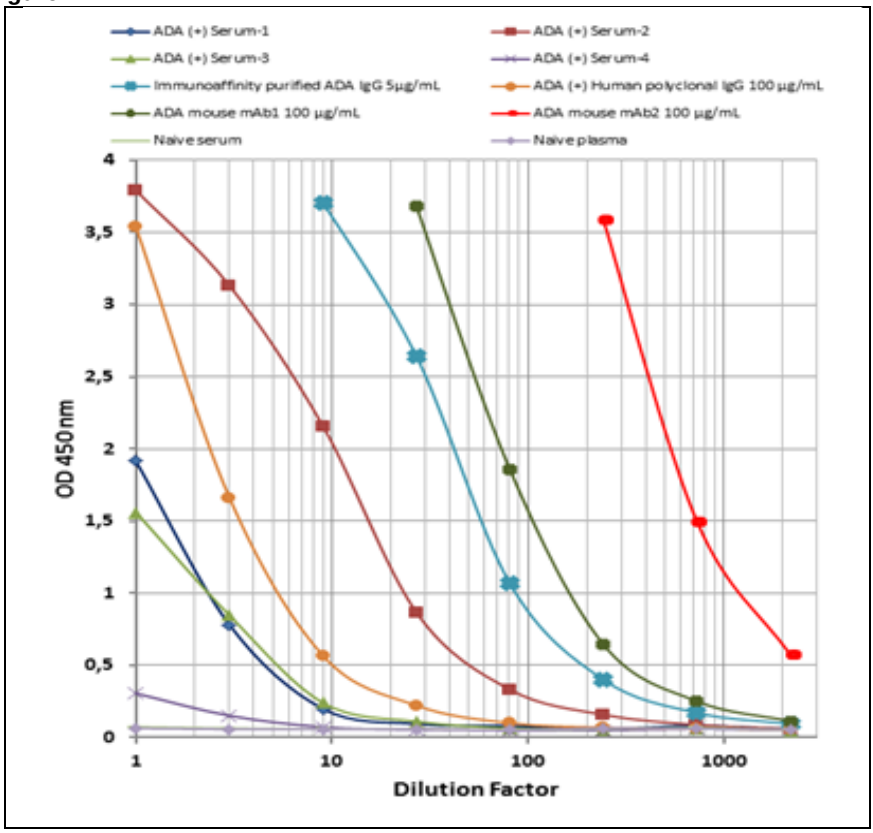
Figure 1.



Generally, the sensitivity of the ADA assay is calculated as the lowest concentration of the positive control that can consistently generate a positive signal. However, as shown in Figure 2 below, data obtained at the *ImmunoGuide* Laboratory, the assay sensitivity also differs significantly depending on the high vs low affinity antibody used for the construction of the standard curve in the assay system. For example, when an immunoaffinity-purified ADA was used, it was observed that the lowest detectable level that can be clearly distinguished from the negative control value is somewhere around 10ng/mL. It is much more lower when mAb2 is used instead.

In any case this number is highly depending on the characteristics of the ADA under investigation. Therefore this number may be significantly different for each individual sample under investigation. Therefore, it is best to keep up using the same ADA for constructing the standard curve.

Figure 2.



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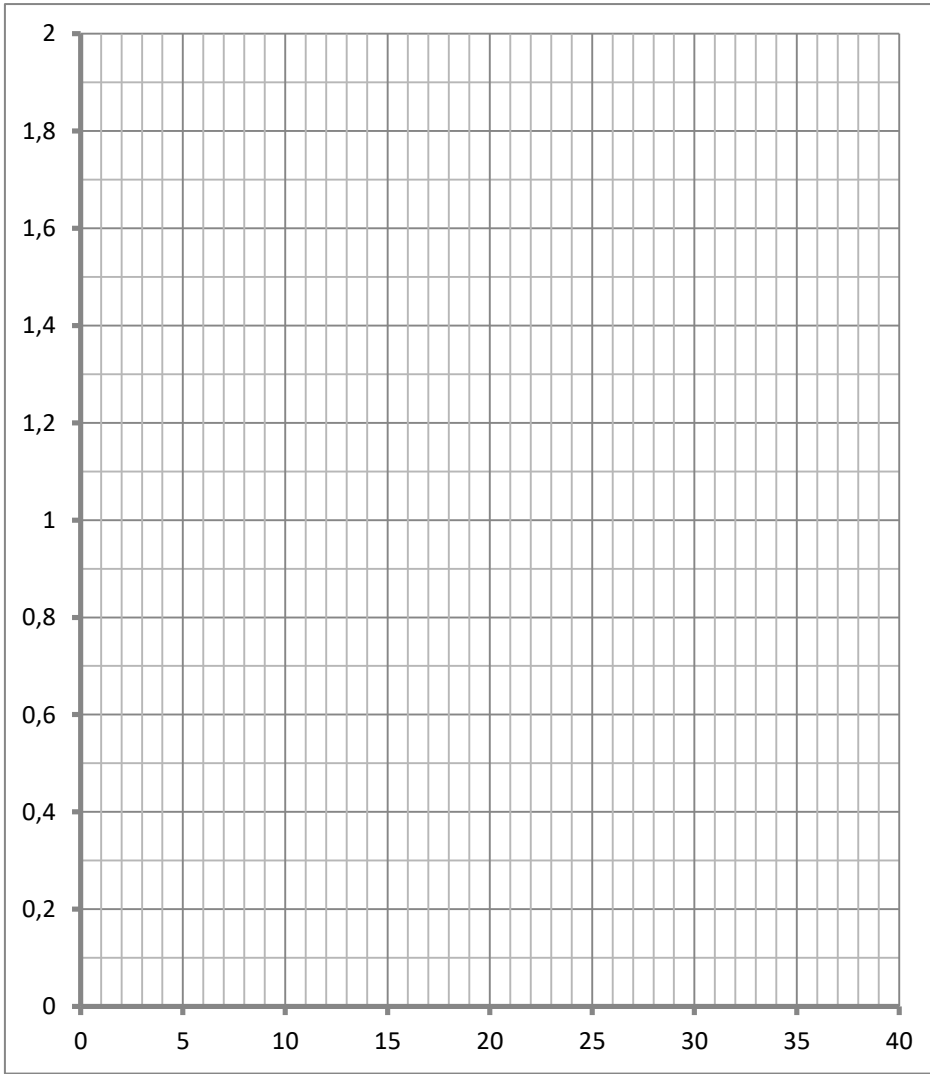
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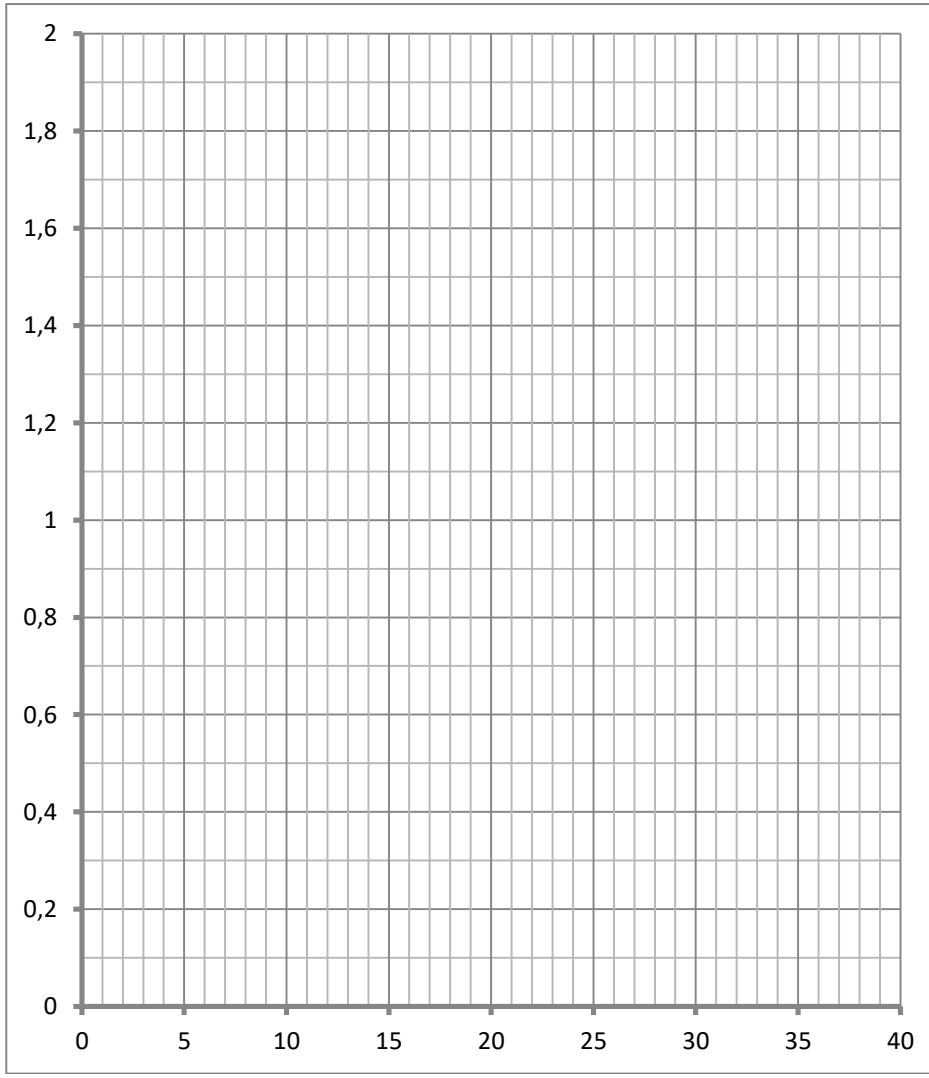
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