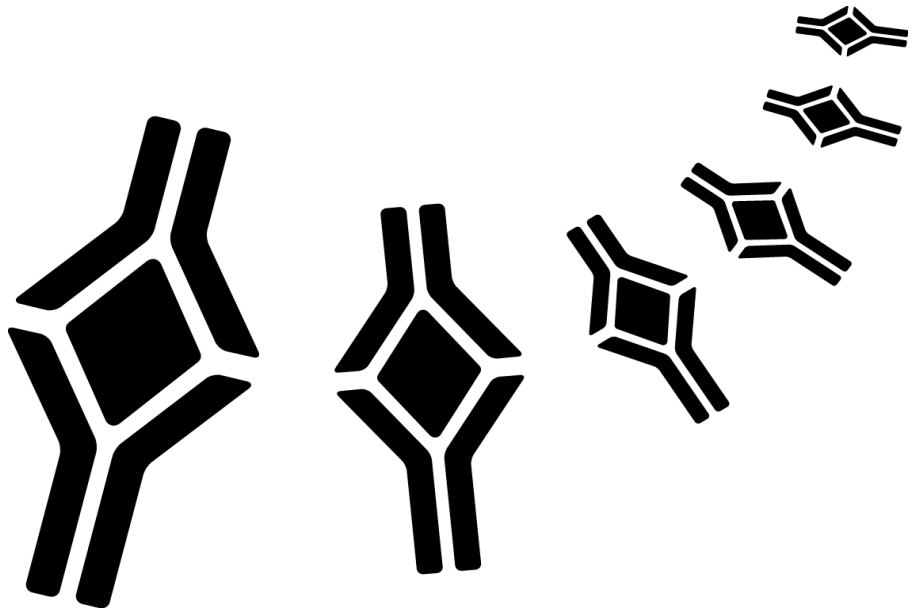


HUMAN MICA ELISA

Product Data Sheet

Cat. No.: RGP026R

For Research Use Only

VERSION 51 131011 46

CONTENTS

» **This kit is manufactured by:**
BioVendor – Laboratorní medicína a.s.

» **Use only the current version of Product Data Sheet enclosed with the kit!**

1. INTENDED USE

The MICA ELISA is to be used for research use only quantitative determination of MHC class I chain-related gene A glycoprotein, in cell culture supernatants, buffered solutions. **This kit has been configured for research use only and is not to be used in diagnostic procedures.**
Special note for serum and plasma quantification: high detection level is found in human serum and plasma samples. The appearance of a high signal could be the result of the matrix and/or interaction with other molecules. Consequently serum and plasma quantification is under the responsibility of the user.

2. INTRODUCTION

MICA is a transmembrane glycoprotein that functions as a ligand for human NKG2D, an activating receptor expressed on NK Cells, NKT Cells, dg Tcells and CD8+ba T Cells (1). Recognition of MICA by NKG2D results in the activation of cytolytic activity and/or cytokine production by these effectors cells. MICA recognition is involved in tumour surveillance, viral infections, and autoimmune diseases.
Major histocompatibility complex (MHC) class I chain-related gene A and B (MICA and MICB) are transmembrane glycoproteins that function as ligand for NKG2D. These two proteins possess three extracellular immunoglobulin-like domains, but have no capacity to bind peptide or interact with b2-microglobulin. The genes encoding MICA/B are found within the MHC on human chromosome 6.
MICA and MICB have no role in antigen presentation but fonction as signal of cellular distress and interact with NKG2D-DAP10, the activating receptor. (2) They are frequently expressed in ephitelial tumor and may promote anti tumor NK and T-cell response (1).
Intestinal cells express MICA/MICB which are up-regulated under stress and in many gastrointestinal tumors. Release of MIC molecules from the cell surface is thought to constitute in immune escape mechanism of tumor cells (3).
MICA/MICB expression is elevated in the sera of patients with colorectal carcinoma (4) and widely expressed in prostate carcinoma:
MICA/MICB may be a novel biomarker for prostate cancer (5) and its expression is also monitor in Crohn's disease. (6)

3. PRINCIPLE OF THE METHOD

The MICA Kit is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA). A monoclonal antibody specific for MICA has been coated onto the wells of the microtiter strips provided. Samples, including standards of known MICA concentrations and unknown are pipetted into these wells.
During the first incubation, the MICA antigen is added to wells. After washing, a biotinylated monoclonal antibody specific for MICA is incubated. Then the enzyme (streptavidin-horse radish peroxydase) is added. After incubation and washing to remove all unbound enzyme, a substrate solution of the bound enzyme is added to induce a coloured reaction product. The intensity of this coloured product is directly proportional to the concentration of MICA present in the samples.

4. REAGENTS PROVIDED AND RECONSTITUTION

REAGENTS (Store at 2-8°C)	**COLOUR CODE**	**Quantity**	**State**
Antibody Coated Microtiter Strips		96 wells	Ready to use
Plastic cover		2	
Standard: 5000 pg/ml	Yellow	2 vials	Reconstitute with the volume of standard diluent indicated on the vial. (See Reagents Preparation)
Standard Diluent buffer	Black	1 vial (30 ml)	10X concentrate. Dilute in distilled Water.
Biotinylated anti-MICA	Red	1 vial (0.4 ml)	Dilute in biotinylated antibody diluent
Biotinylated Antibody Diluent	Red	1 vial (7 ml)	Ready to use
Streptavidin-HRP		2 vials (5 µl)	Add 0.5ml of HRP-Diluent before further dilutions
HRP Diluent	Red	1 vial (23 ml)	Ready to use
Washing Buffer	White	1 vial (10 ml)	200X concentrated. Dilute in distilled Water
Chromogen TMB:		1 vial (11 ml)	Ready to use
H_2SO_4: Stop Reagent	Black	1 vial (11 ml)	Ready to use

5. MATERIAL REQUIRED BUT NOT PROVIDED

- Distilled water.
- Pipettes : 10 µl, 50 µl, 100 µl, 200 µl and 1000 µl.
- Vortex mixer and magnetic stirrer.

6. SAFETY

- For research use only.
- Avoid any skin contact with H_2SO_4 and TMB. In case of contact, wash thoroughly with water.
- Do not eat, drink, smoke or apply cosmetics where kit reagents are used.
- Do not pipette by mouth.

7. PROCEDURAL NOTES/LAB. QUALITY CONTROL

1 When not in use, kit components should be stored refrigerated or frozen as indicated on vials or bottles labels. All reagents should be warmed to room temperature before use. Lyophilised standards should be discarded after use.
2. Once the desired number of strips has been removed, immediately reseal the bag to protect the remaining strips from deterioration.
3. Cover or cap all reagents when not in use.
4. Do not mix or interchange reagents between different lots.
5. Do not use reagents beyond the expiration date of the kit.
6. Use a clean disposable plastic pipette tip for each reagent, standard, or specimen addition in order to avoid cross-contamination; for the dispensing of H_2SO_4 and substrate solution, avoid pipettes with metal parts.
7. Use a clean plastic container to prepare the washing solution.
8. Thoroughly mix the reagents and samples before use by agitation or swirling.
9. All residual washing 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.
10. The TMB solution is light sensitive. Avoid prolonged exposure to light. Also, avoid contact of the TMB solution with metal to prevent colour development. Warning TMB is toxic avoid direct contact with hands. Dispose off properly.

11. If a dark blue colour develops, this indicates that the TMB solution has been contaminated and must be discarded. Read absorbances rapidly after completion of the assay.
12. When pipetting reagents, maintain a consistent order of addition from well-to-well. This will ensure equal incubation times for all wells.
13. Respect incubation times described in the assay procedure.

8. SPECIMEN COLLECTION, PROCESSING AND STORAGE

Cell culture supernatants - Remove particulates and aggregates by spinning at approximately 1000 g for 10 min.
Cell lysats - After spinning at approximately 400 g for 5 min, remove the supernatant and wash once again with PBS. Suspend cells in a cold lysis buffer. After 30 min of incubation, carefully remove the supernatant after spin at 10000 g for 10 min at 4°C. Store at -70°C.
Storage - If not analysed shortly after collection, samples should be aliquoted (250-500 µl) to avoid freeze-thaw cycles and stored frozen at –70°C. Avoid multiple freeze-thaw cycles of frozen specimens.
When possible, avoid use of badly hemolyzed or lipemic sera. If large amounts of particles are present, this should be removed prior to assay by centrifugation or filtration.
Recommendation: Do not thaw by heating at 37°C or 56°C. Thaw at room temperature and make sure that sample is completely thawed and homogeneous before assaying.

9. PREPARATION OF REAGENTS

9.1 Standards

Standard have to be reconstituted with the volume of standard buffer diluent indicated on the vial. This reconstitution produces a stock solution of 5000 pg/ml MICA. Serial dilutions of standard must be made before each assay and cannot be stored.

9.2 Dilution of biotinylated anti - MICA

Preparation immediately before use is recommended. Dilute the biotinylated anti-MICA with the biotinylated antibody diluent in a clean glass vial according to the number of wells to be used. See the next table for volumes to pipette. Extemporaneous preparations are recommended.

Number of Wells used	Biotinylated Antibody (µl)	Biotinylated Antibody Diluent (µl)
16	40	1040
24	60	1560
32	80	2120
48	120	3180
96	240	6360

9.3 Dilution of Streptavidin-HRP

Add 0.5 ml of HRP diluent to a 5 µl vial of Streptavidin-HRP. DO NOT KEEP THIS DILUTION FOR FURTHER EXPERIMENTS.

Dilute immediately before use. Following the number of wells to be used, further dilutions of Streptavidin-HRP should be made with HRP diluent in a clean glass vial: see hereafter the table for volumes to pipette.

Number of Wells	Streptavidin-HRP(µl)	Strep-HRP Diluent (ml)
16	30	2
24	45	3
32	60	4
48	75	5
96	150	10

9.4 Washing Buffer 200X concentrate

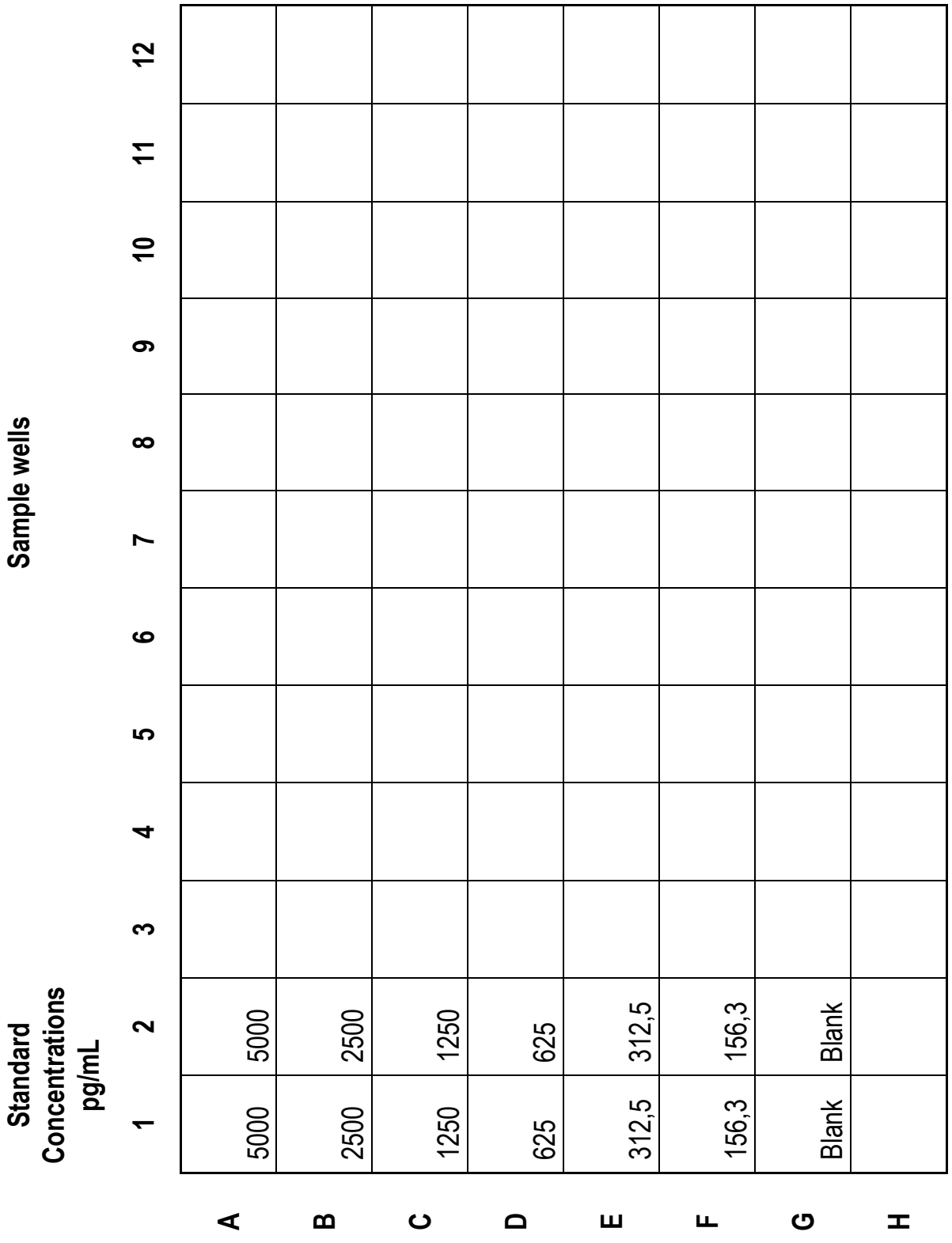
Dilute 200 times in distilled water.

10. ASSAY METHOD

a) Before use, mix all reagents thoroughly without making foam.
b) Determine the number of microwell strips required to test the desired number of samples, plus appropriate number of wells needed for running blanks and standards. Each sample, standard and blank should be assayed **in duplicate** . Remove sufficient microwell strips from the pouch.
c) Add 100 µl of standard diluent (see preparation of reagents) to standard wells B1, B2, C1, C2, D1, D2, E1, E2, F1, F2. Reconstitute standard vial with the appropriate volume as described in the chapter reagents preparation. Pipet 200 µl of standard into wells A1 and A2 (see Plate Scheme below). Transfer 100 µl from A1 and A2 to B1 and B2 wells. Mix the contents by repeated spirations and ejections. Take care not to scratch the inner surface of microwells. Repeat this procedure from the wells B1, B2 to wells C1, C2 and from wells C1, C2 to D1, D2 and so on creating two parallel rows of MICA standard dilutions ranging from 5000 to 156.3 pg/ml. Discard 100 µl from the content of the last microwells used (F1, F2). Alternatively these dilutions can be done in separate tube and diluted standard pipetted directly into wells.
d) Add 100 µl of standard diluent to the blank wells (G1-G2).
e) Add 100 µl of sample to sample wells.
f) Cover with a plate cover and incubate for 2 hours at room temperature (18°C - 25°C).

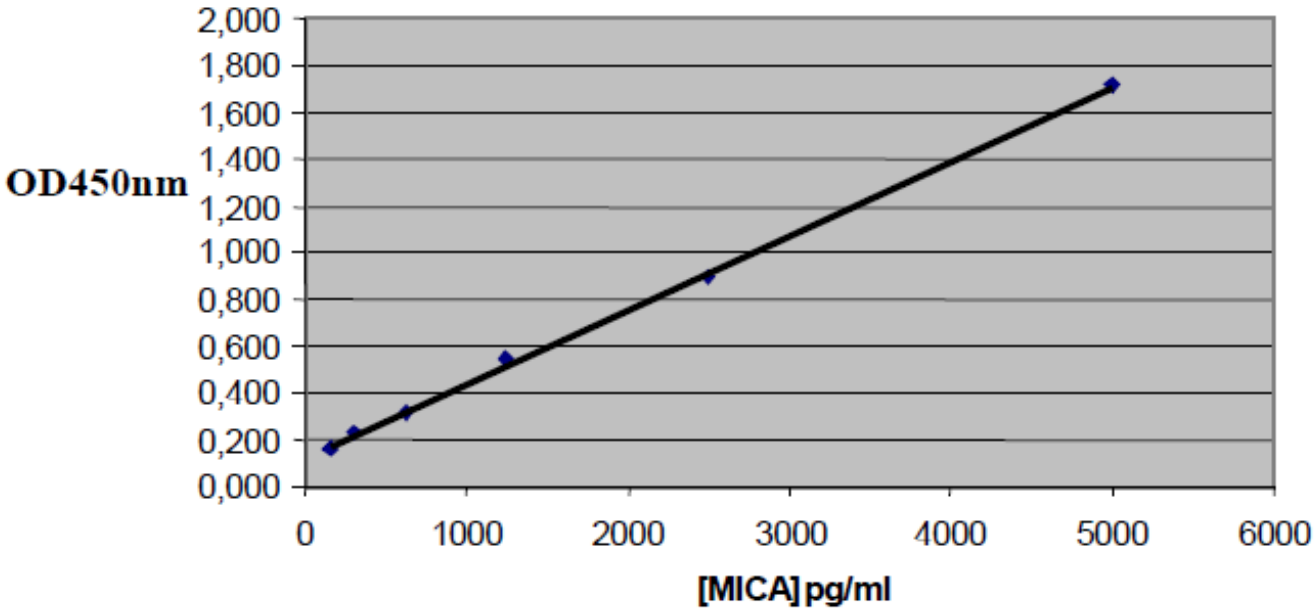
g) Remove the cover and wash the plate as follows:
   1) aspirate the liquid from each well;
   2) dispense 0.3 ml of washing solution into each well;
   3) aspirate again the content of each well;
   4) Repeat steps 2) and 3) two times.
h) Preparation of biotinylated anti-MICA: (see preparation of reagents).
i) Add 50 µl of diluted biotinylated anti-MICA to all wells.
j) Cover and incubate 1 hour at room temperature.
k) Wash as described in point g)
l) Prepare HRP solution just before use: (see preparation of reagents).
m) Dispense 100 µl of HRP solution into all wells, including the blank wells. Put back the cover.
n) Incubate the microwell strips at room temperature for 30 minutes.
o) Remove plate cover and empty wells. Wash microwell strips according to point g). Proceed immediately to the next step.
p) Pipette 100 µl of ready-to-use TMB substrate solution into all wells, including the blank wells and incubate in the dark for 5-15 min minutes at room temperature. Avoid direct exposure to light by wrapping the plate in aluminium foil. Incubation time of the substrate solution is usually determined by the ELISA reader performances: many ELISA readers record absorbance only up to 2.0 O.D. The O.D. values of the plate should be monitored and the substrate reaction stopped before positive wells are no longer properly readable.
q) The enzyme-substrate reaction is stopped by quickly pipetting 100 µl of H_2SO_4: stop reagent into each well, including the blank wells, to completely and uniformly inactivate the enzyme. Results must be read immediately after the addition of H_2SO_4: stop reagent.
r) Read absorbance of each well on a spectrophotometer using 450 nm as the primary wavelength and optionally 620 nm (610 nm to 650 nm is acceptable) as the reference wavelength.

11. SUGGESTED PLATE SCHEME



12. DATA ANALYSIS

Generate a linear standard curve by plotting the average absorbance on the vertical axis versus the corresponding MICA standard concentration on the horizontal axis. The amount of MICA in each sample is determined by extrapolating OD values to MICA concentrations using the standard curve.



Typical MICA standard curve ranging from 156 pg/ml to 5000 pg/ml

13. LIMITATIONS OF THE PROCEDURE

Do not extrapolate the standard curve beyond the 5000 pg/ml standard curve point. The dose-response is non-linear in this region and good accuracy is difficult to obtain. Concentrated samples (> 5000 pg/ml) have to be tested with some dilutions with standard diluent or with your own sample buffer. Sample MICA concentration could be assess without dilution of samples. If there's a dilution (e.g. ″, multiply results by the appropriate dilution factor).
The influence of various drugs, aberrant sera (hemolyzed, hyperlipidemic, jaundiced...) has not been investigated. The rate of degradation of native MICA in various matrices has not been investigated.

14. PERFORMANCES AND CHARACTERISTICS

14.1 Sensitivity

The minimum detectable dose of MICA is 123 pg/ml.
This has been determined by adding 3 standard deviations to the mean optical density obtained when the zero standard was assayed 32 times in duplicates.

14.2 Precision

	Intra-Assay				Inter-Assay				
Sample	**n**	**Mean (pg/mL)**	**SD**	**CV%**	**Sample**	**n**	**Mean (pg/mL)**	**SD**	**CV%**
A	6	2214,6	50,3	2,3	A	18	2416	187,1	7,7
B	6	1102,1	39,5	3,6	B	16	1200	105,2	8,8
C	6	641,6	47,7	7,4	C	18	627	50,9	8,1

14.3 Specificity

The assay recognises recombinant and natural MICA. There's no cross reactivity with IL-7, IL-12p40, IL-2R, Trail, CD31, IL-13Ra2, IL-5, gp130, TNFa.

14.4 Recovery

We obtained a recovery of 92% for MICA concentration when we add MICA in a serum depleted of MICA.

15. REFERENCES

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5. Prevalent expression of the immunostimulatory MHC class I chain-related molecule is counteratcted by shedding in prostate cancer.Jennifer D.Wu. J of clinical investigation, 2004, Vol 114 :560-568
6. CD4+NKG2D+ T cells in Crohn's disease mediate inflammatory and cytotoxic responses through MICA interactions. Allez M., Gastroenterology. 2007; 137 (7) 2346-58.

16. ASSAY PROCEDURE SUMMARY

Total procedure length 3 h 45 mn

Add 100 µl of sample or diluted standard

⇓

Incubate 2 hours at room temperature

⇓

Wash three times

⇓

Add 50 µl of biotinylated detection antibody

⇓

Incubate 1 hour at room temperature

⇓

Wash three times

⇓

Add 100 µl of Streptavidin-HRP

⇓

Incubate 30 min at room temperature

⇓

Wash three times

⇓

Add 100 µl of ready-to-use TMB
Protect from light. Let the color develop for 5-15 min.

⇓

Add 100 H_2SO_4

⇓

Read Absorbance at 450 nm

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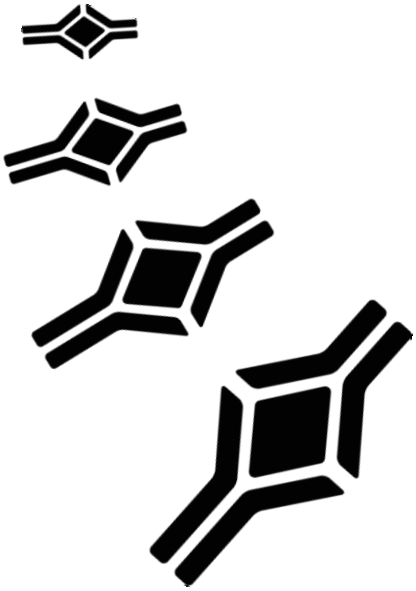

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