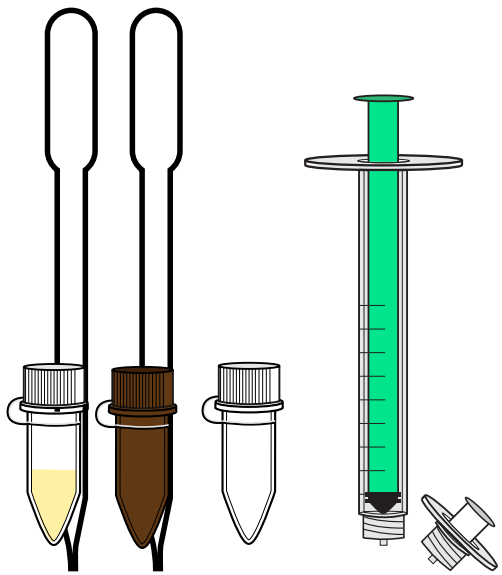
GENETIC ENGINEERING WITH THE

# Extract-it Kit - Liquid Culture™

## User Manual



Make with biology

amino labs

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# Table of Content - Extract-it Kit (Liquid Culture)

# Welcome! Let's get started

This User Guide was created to help you get the most out of your Amino Labs kit.  Even if you are familiar with bioengineering, science or other Amino Labs™ product, please take the necessary time to read through this guide. This will ensure you practice safe science, get the most out of your Extract-it Kit™ and finally, know what to do in case of a spill or accident!

This manual contains the step by step instructions on how to run your experiment. Make sure to follow our tips! We will also cover "what's next", how to keep your creations, left over ingredients and clean up.

Following this guide will help ensure that you are getting the most out of your current and future experiences to keep on making new creations with DNA.  Amino Labs is very excited to welcome you to the world of the biotechnology with our ecosystem of easy-to-use, easy-to-succeed at products!

Have fun!

# Practice Safe Science

**Genetic engineering and life sciences are safe activities when you follow simple guidelines. Read on to ensure you adopt safe practices.**

The kit in your hands contains only non-pathogenic ingredients. These are part of the biosafety Risk Group 1 (RG1) (Biosafety Level 1). This is the most benign level and therefore the safest: with these kits, no special containment or training is required in North America. But you must follow these safety guidelines for your safety and the success of your experiment(s)!

**We recommend the system and kits for ages 12+, under adult supervision, and 14+ with or without supervision.** We recommend that an adult empties the discard container. The cleaning instructions must be strictly followed for safety and experiment success. Make sure to store the kit per the instructions found in this booklet.

- **Do not eat or drink near your experiments.** Keep your experiment at least 10 feet from food, drinks, ***etc***. Under no circumstances should you eat any of the kit's content.
- **Immunocompromised persons:** While the ingredients in these kits are non-pathogenic, some persons, such as immunocompromised persons, can be affected by large numbers of bacteria and should talk to their doctor before doing any experiment.
- **Wash your hands before and after** manipulating your experiment, or the hardware.
- **Wear gloves**, even when cleaning your station or handling the kit contents (petri plates, loops, etc). This will protect you from your experiment, and your experiment from you. Any latex, nitrile, or general purpose gloves you can find at the pharmacy will do. After you put your gloves on, be aware of what you touch. Try not to touch your face, scratch itches with your gloved fingers!
- **If using the DNA Playground™ or BioExplorer™ place it on a stable work surface.** Keep it level at all times.
- **Clean up your station, spills and work surface before and after use.** Use a 10% solution of chlorinated bleach generously sprayed onto a paper towel and rub onto any contaminated surfaces. (Careful! This can discolor your clothes). A chlorinated spray cleaner also works.
- **Find a container to hold the inactivation bag where you will discard used consumables**. An old 1L yogurt container, large plastic cup or the like will do. Used consumables will be loops, any tube or used petri dish.
- **Eye-wear is not provided but can be worn**.

You can download a biosafety poster for your space from www.amino.bio/biosafetyinaction and complete a short safety quiz at www.amino.bio/biosafety-quiz

If you would like to do a short Online lab safety course for your edification, we recommend a Government of Canada course: www.amino.bio/biosafety

# Equipment Needed

To use the Extract-it Kit you will need the following:

- **Microcentrifuge**
  Spins at 13,000 RPM or greater
- **Large tube Centrifuge (Optional)**
  Spins up to 4,000 RPM

# Cleaning and safety supplies needed

- **Disposable container 500ml-1L** for waste (e.g. yogurt container )
- Nitrile, (or similar) **gloves** like the ones found at a pharmacy.
- **Chlorinated bleach** (*mix a 10% solution: 1 part bleach to 10 parts water*) and a plastic bag to clean up.

# Setting up your space

Setting up your space is easy. Find a level, non-porous surface. Make sure the surface is bleach safe, and wipe it down with a solution of 10% chlorinated bleach or a chlorinated cleaner. Find and place a container of about 500ml-1L to collect your experiment waste : liquids, ingredient tubes, wipes. We suggest an old yogurt container or similar that you can fill with bleach to inactivate and then throw out at the end.

Make sure your centrifuge(s) is on a solid, level surface. Refer to the manufacturer's instructions for set up.

# What does your kit allow you to do?


The Extract-it kit (liquid culture) allows you break open pigment-producing bacteria that you cultured in a shaker incubator or in Amino Labs' Bioexplorer. By breaking open the bacteria you engineered to create pigment, you will then be able to collect this pigment to use it for art! This is the same process by which you would create medicine, flavours, fragrances and more using engineered bacteria. The next step when bio-manufacturing medicine, flavours and fragrances would be the purification and quality control of them. Since we are making pigments for art or simply to look at, we do not need to worry about purifying them to such a degree. What we will do with this kit is filter-sterilize them to remove any bacteria debris.

First, the bacteria's cell wall will be broken open through a process called lysis and then the solution will be centrifuged so that you can collect a liquid made out of your pigment-producing  proteins. You will then filter this liquid to help sterilize it. What's great about this is that the DNA program is still present within the product, so that if someone ever wanted to, they could copy it from there, and grow it once more in bacteria!

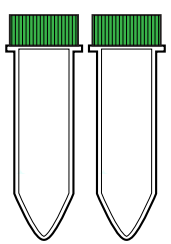
# What's in your kit?

Inside the Extract-it Kit™, you will find these components:

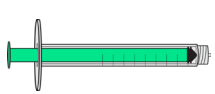
**Lysis buffer: :** softly breaks open (lyses) the cells to release the cell contents. This buffer should be used in concert with Lysis Accelerator.

**Lysis Accelerator:** includes enzymes that break down the cell wall of bacteria and works with Lysis Buffer to release the contents of cells into their environment.

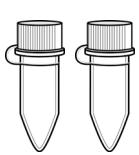
**0.22 um filter** : This filter has pore sizes that are 0.22 um which are smaller than bacteria. This means bacteria cannot pass through, but your pigment (smaller than 0.22 um) can.



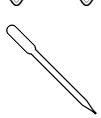
**Collection Tubes :** These 50mL tubes are used to collect and pellet cells from the Bioproduction Lab using a centrifuge. If you don't have a large centrifuge that can fit these tubes, you can also add cells to the tube and let them sit in the fridge for 24-48 hours and the bacteria will settle at the bottom



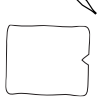
**Syringe:** Used to push unfiltered extract through a filter. Caution! Do not press to hard to avoid liquid mishaps. Goggles recommended when using the syringe.



**(2)1.5 mL Screw Cap Tube :** Use one to store your final, extracted and filtered product, and one to as a cell debris spin tube.



**Pipet :** Pipets are used to re-suspend the cells in Lysis Buffer

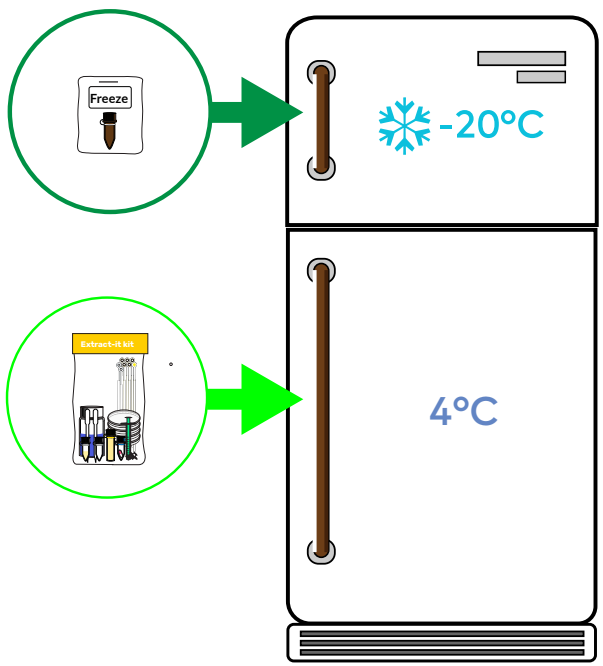


**Burst bag:** a plastic bag to use over the syringe-filter sterilization to minimize possible mess.

# Unpacking and Storing your kit

For a better shelf life and successful experiments:

- place your Extract-it Kit™ bag in a standard refrigerator at around 4°C.
- place the smaller Freezer bag with the brown tube(s)  in a freezer.




**Do Not Freeze all of your kit!**

**Do not leave tubes at room temperature!**

# Technical Specifications

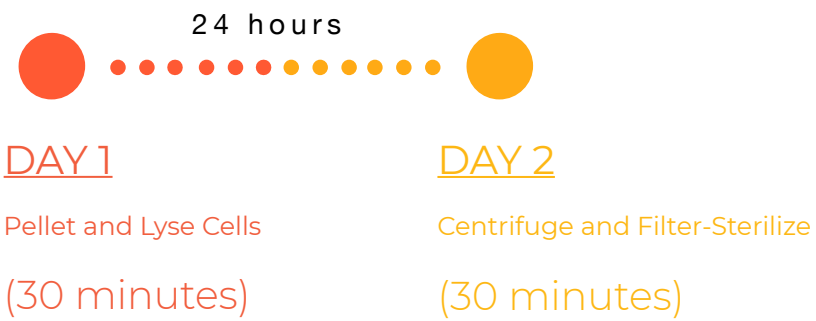
Lysis buffer: 1 mL
Lysis Accelerator: 5 mg

0.22 um syringe filter
3mL syringe

Tubes:
(2) 50 mL
(2) 1.5 mL Screw Cap

# Implementation Timeline

The Extract-it Kit™ takes 2 days of hands-on activity to complete, and 24 to 72 hours to see results.




3 "activities" or steps make up the Extract-it experiment:

1. Collect bacteria and centrifuge it down into a “pellet”.
   **Day 1, 25-35 minutes**

2. Lyse (break open) the bacteria using surfactant and enzymes
   **Day 1, 5 minutes with a 1-24 hour resting period**

3. Collect and filter the pigment
   **Day 2, 30 minutes**

# Start your Experiment: *Experiment protocol*

## 1. Collect & Pellet the engineered bacteria
**25-35 minutes**

**Goal**
Collect bacteria and pellet them.

**You will need**
• Your liquid culture of engineered bacteria, 50 to100 mL
• (2) Collection Tubes
• Optional: Centrifuge for large tubes (4000 RPM)

**Creating your liquid culture**
*To get your liquid culture of engineered bacteria, you can either use the BioExplorer starte kit and follow the instruction manuals for them, or you can create a liquid culture with the engineered cells from the Engineer-it Kit using a shaker incubator, and selective LB media. Contact Amino Labs for help if you need it!*

*Grow your liquid culture for 24-48 hours, until you see the LB media change color - as the bacteria grow and produce their pigment, the yellow LB media of the liquid culture will take on the color of your pigment. Remember your color theory - the color of your pigment will be added to the yellow of the LB media, so if you are growing magenta, raspberry red, or orange pigment-prodcucing bacteria, the liquid culture will turn an orangey-pink color, whereas if you are growing blue, purple, violet, teal pigment-producing bacteria, the liquid culture will be more of a blue/purple-gray. Cyan and yellow producing-bacteria do not affect the visible color of the liquid culture so much, so you will have to look for cloudiness of liquid, or use an blackligh to see the pigment fluoresce.*

*Once the liquid culture is cloudy and as changed color, you are ready to start.*

**Collecting your bacteria**

1.1 Open one of your collection tube and fill it up to the 45 mL line with your liquid culture. This will leave about 5 mL of space at the top to prevent any spills. Be careful not to spill on the counter!

1.2 Fill your second collection tube up to the 45 mL line with tap water. This will become your balance tube *** It is important to make sure the tubes have the same amount of liquid before you centrifuge to ensure the centrifuge will be balanced and safe to use.***

1.3 Place your 2 collection tubes at opposite position in your large centrifuge to balance the rotor and turn it on at 4000 RPM for 10 minutes.

**Note:** If you do not have a 50 mL tube centrifuge, let your collection tube with the liquid culture sit upright on a counter or in a fridge for 24-48 hours, or until you see a collection of colored bacteria at the bottom - this is your bacteria pellet.

1.4 Once your bacteria is collected at the bottom of your tube into what is called a pellet, you will get rid of the liquid on top. This liquid is called the supernatant. Gently pour it into a waste container. Be careful not to disturb your bacteria pellet with sharp movements! If the pellet breaks apart, you can centrifuge your tubes again.

1.5 If you have any remaining liquid culture, you can add it to the collection tube, on top of the existing bacteria pellet, and centrifuge it once more at 4000 RPM for 10 minutes. Remember to match the volume of liquid in your collection tube and balance tube of water to make sure your centrifuge is balanced. Discard any resulting supernatant.

Do not exceed 100 mL of liquid culture in one extraction, as your kit does not contain enough of the lysing ingredients to break open that many cells. In science, more is not always better!

1.6 Once you have collected your liquid culture, you can either inactivate and clean your BioExplorer/Shaker incubator containers, or add more selective LB media to grow more of the same pigment-producing bacteria.

# 2. Lyse the Bacteria
**15 minutes with 12-24 hour wait**

**Goal**
Re-suspend the cells in lysis buffer and enzyme in order to break down the cell wall and release the product

**Materials from your kit**
• Your collection tubes from previous step
• (1) Lysis Buffer tube
• (1) Lysis Accelerator tube (it would have been placed in the freezer upon arrival of the kit)
• (1) 1 mL Pipet
• (1) Balance tube
• (1) 1.5 mL tube

2.1 Once you have a bacterial pellet and all of your supernatant is poured off into a waste container, take your 1 mL tube of Lysis Buffer and pour it into the collection tube with your pellet. With a pipet, pipet up and down until the bacteria pellet breaks apart and the bacteria become fully suspended in the lysis buffer * Try not to create too many bubbles! *

2.2 Take the lysis buffer with suspended bacteria and pour or pipet it into the brown tube with Lysis Accelerator, put the lid on, and invert it 10 times to mix.

2.3 Set the tube upright on your lab area (you can use a tube rack if you have one, or lean it against some of your lab supplies) and let incubate at room temperature for 12-24 hours. During this period, the bacteria will be broken open and the pigments will be released into the solution. The longer the incubation time, the more product you will extract. If you have the opportunity , invert the tube to mix during the incubation period. This will improve the lysis reaction!

2.4 **After your 12 to 24 hour incubation period is over,** pour the solution into one of the 1.5 mL tube. Since this tube is transparent, it will allow you to keep an eye on your solution in the next step.

2.5 Take your balance tube and verify that the volume of liquid in it and in your solution tube are the same. Add or remove water from the balance tube as needed.

2.6 Place your tubes in your microcentrifuge in opposite position to ensure the microcentrifuge is balanced. Spin the tubes at 13,000 RPM for 15 minutes.
*** Immediately move to the step 3 ***

# 3. Collect & Filter the Product
**10-15 minutes**

**Goal**
Passing the extracted pigment through a 0.22 um filter to get rid of cells and other debris (sterilize the products)

**Materials from your kit**
• Your microcentrifuged solution tube from the previous step
• (1) Syringe
• (1) Syringe Filter
• (1) 1.5 mL tube for final pigment
• (1) Burst bag

3.1 Take your syringe and syringe filter, and attach the syringe filter to the end of the syring by twisting it on. Avoid touching the ends of the syringe filter!

3.2 Place the syringe inside the burst bag so the the tip of the syringe filter pokes out of the hole at the bottom of the burst bag, and cover the syringe with the burst bag, leaving the top of the bag open.

The burst bag is here to help contain any liquid that could spray out from where the filter and syringe meet if you push on the syringe too hard or too fast in the next step. Do the next steps carefully and gently, making sure your burst bag is over this area!

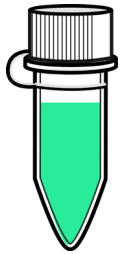
3.3 Place your syringe filter end over your open 1.5 mL final pigment tube. This tube will collect the filtered pigment solution. Make sure the final pigment tube is steady - use a tube holder to hold it upright, or ask a someone for help holding it.

3.4 Remove the plunger from the syringe, and gently pour the supernatent from your microcentrifuged solution tube into the syringe. ***This solution should be a translucid, colored solution. It should not cloudy. If it is cloudy, transfer it back to the tube and centrifuge for another 15 min. The cloudiness is cell debris and will clog the filter irreversibly.***

3.5 Gently put the syringe plunger back into the syringe and slowly press down to pass the pigment solution through the filter and into the final pigment tube.
***Be careful not too press to hard, because the filter could explode. If you did not effectively centrifuge the cell debris, the filter could get clogged.***

3.6 Once all your solution is filtered, close your final pigment tube and place the rest of the items in your discard container. You now have filtered sterilised bio-engineered pigments!

# Congratulations!



Using the Extract-it Kit™, you extracted and filter-sterilised pigment-producing proteins created by engineered bacteria, just like scientist and industries do every day inside their large laboratories.

Your extracted pigments are stable at room temperature, and will fluoresce under blacklight if they were created with cyan, magenta, yellow or raspberry red pigment-producing bacteria. It is always best to keep them out of direct sunlight as they could become sunbleached. Use them to paint or make art, or keep them as a memento of your scientific accomplishements!

Congratulations! We hope you enjoyed the experience, and will continue your journey in biotechnology.

## Storage, inactivation and clean up

After your experiment, you will have engineered bacteria, used loops, pipets, syringe(s), filter, and tubes:

1. Inactivate and dispose of all of your used items, tubes, syringes, etc, by adding 1 part chlorinated bleach to 5 parts water. (If you have the inactivation bag, follow the instructions on the label.)

2. Make sure to inactivate and clean your BioExplorer or shaker incubator equipment.

3. Use a solution of 10% bleach or spray cleaner to wipe down your work area and equipment.

# Glossary

**Agar:** is a Jello-like substance that serves as a growth media for bacteria. It is mixed with our bacteria's favorite food: Lysogeny broth (LB). LB is made up of yeast, vitamins, and minerals. LB can also be found liquid-form.

**Antibiotics:** When you transform bacteria, they will become resistant to a type of antibiotics no longer used in hospitals. This antibiotic will be mixed in with the agar and LB so that, as you incubate your culture, only transformed bacteria will grow. This is called a "selection marker".

**Buffers:** Buffers are saline solutions that help, in this case, open up the cell membranes so that they may take up new DNA.

**Cells:** Cells are tiny, living units that function like mini-factories. Bacteria are single-celled organisms (unicellular) microorganisms. They are different from plant and animal cells because they don't have a distinct, membrane-enclosed nucleus containing genetic material. Instead, their DNA floats in a tangle inside the cell. Individual bacteria can only be seen with a microscope, but they reproduce so rapidly that they often form colonies that we can see. Bacteria reproduce when one cell splits into two cells through a process called binary fission. Fission occurs rapidly, in as little as 20 minutes.

**Competent Cells:** Since DNA is a very hydrophilic molecule, it won't normally pass through a bacterial cell's membrane. In order to make bacteria take in the DNA plasmid, the cells must first be made "competent" to take up DNA. This is done by creating small holes in the bacterial cells by suspending them in a solution with a high concentration of calcium (the transformation buffer). DNA can then be forced into the cells by incubating the cells and the DNA together on ice, placing them briefly at 42°C (heat shock), and then putting them back on ice. This causes the bacteria to take in the DNA and is called "Transformation".

**DNA:** The DNA is the set of instructions that tells the cell how to function, like a computer program does.

**DNA plasmid:** A plasmid is a small circular piece of DNA (about 2,000 to 10,000 base pairs) that contains important genetic information for the growth of bacteria. Bacteria share vital information by passing it among themselves in the form of genes in plasmids. By inserting a new plasmid in our bacteria, we can get them to produce things for us,

like mini factories. In this case, we have a plasmid that encodes for the creation of colorful pigments.

**Heatshock:** When the cells are moved from ice-cold to warm temperature, typically 42C, in order to take in DNA plasmids more efficiently.

**Inoculation**: when you introduce bacteria into a medium suitable for its growth.

**Inoculating Loops**: Inoculating loops are used to transfer liquids, cells and DNA from one vial to the next instead of traditional lab pipettes, making your job easier, and less costly. They come in different pre-calibrated sizes so you do not need to worry about minuscule liquid volumes. They are also used to spread bacteria on an agar surface without puncturing the soft agar.

**Non-Selective**: A non-selective plate means that any cells /bacteria put on this agar will grow as long as they are oxygen-loving organisms (called aerobic bacteria).

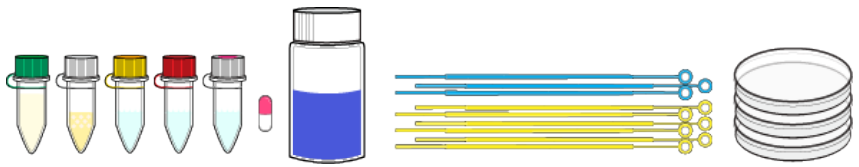
**Plates (or petri dish)**: A petri dish is a small plastic container used to culture (grow) bacteria in a controlled environment.

**Recovery period:** is the period after the heat shock in which the cells develop their antibiotics resistance and start dividing.

**Selective:** A selective plate means it contains antibiotics. When you insert a new DNA program into cells to make them create pigments, or anything else, you also put a “selective marker” (antibiotics resistance) inside the code. This means that only the cells that have taken up the new program will be able to grow on a plate that has the antibiotics mixed in: You only get the cells you transformed!

**Transformation**: See competent cells.

# Troubleshooting



**Your agar is too wet/ doesn't solidify:** The agar, if done correctly, will be the consistency of jell-o. If not:
1. You might not have added all the powder from the tube, resulting in too much water vs. LB agar powder.
2. You may not have fully dissolved the powder, meaning it cannot turn into a gel, and will look cloudy. You can practice by making jell-o! Next time heat and swirl longer to ensure the powder is fully dissolved.

**You don't have any colonies and its been 24+ hours**: Don't worry, every scientist has experienced this, and it can take some practice before success.
1. Double check that your incubator is on at 37°C (if not, or if you are growing at room temperature, then it can take much longer to see the bacteria colonies. Keep waiting!)
2. You may need to try again to hone your skills. See our Youtube videos for tips and tricks on how to improve your chances of success.

**Your colonies of bacteria grew, but they are not colored as per your DNA program / There is mold on your petri plate:** Danger! If at the end of 24-48 hours your resulting bacteria/plate is: i) not the right color; ii) not colorful at all; iii) is black when it shouldn't be, then this is a sign that your culture is NOT YOUR ENGINEERED BACTERIA. You should immediately inactivate it and clean your space and unit.

Pour 100% chlorinated bleach into the dish, put the lid on and let it sit for 24 hours before throwing it out: The strong oxidizing environment degrades any living organisms. After 24 hours, if there are still organisms present add more concentrated bleach until it is almost full, and let stand for a further 24 hours. *Always be aware that concentrated bleach is a strong oxidizing agent and if poured on skin can cause irritation, and on clothes remove color. Follow the safety and handling protocol on the manufacturer's label.*

There may be mold in your environment. We recommend, getting a small air purifier with a HEPA filter for the room.

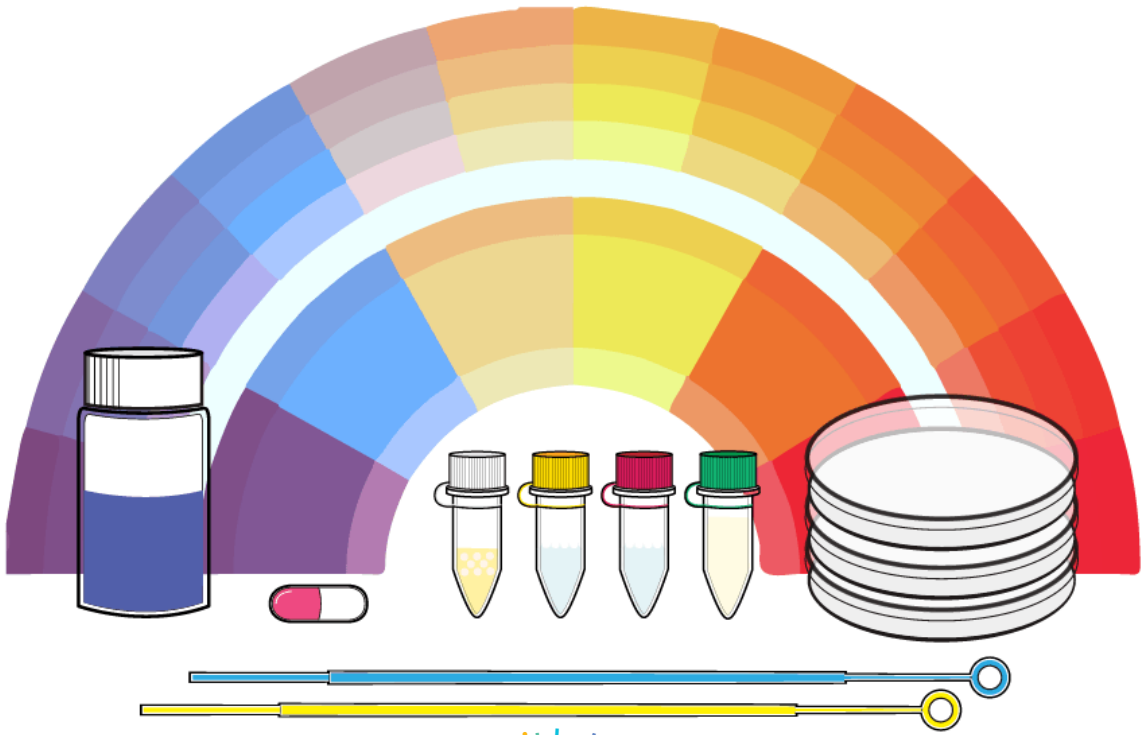
**If anything else causes you issues, please contact us :** help@amino.bio

Made in Canada

All Amino Labs products, from the Hardware to the DNA, are developed, designed, manufactured and packaged by us in our laboratory and workshop in Canada.

We'd love to hear your thoughts, feedback and suggestions so that we can continue to make our products better, and fitting to your needs.  Answers to your questions and help are also just an email away:

Help and General inquiries:  help@amino.bio
Feedback, Suggestions, Comments:  j@amino.bio



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