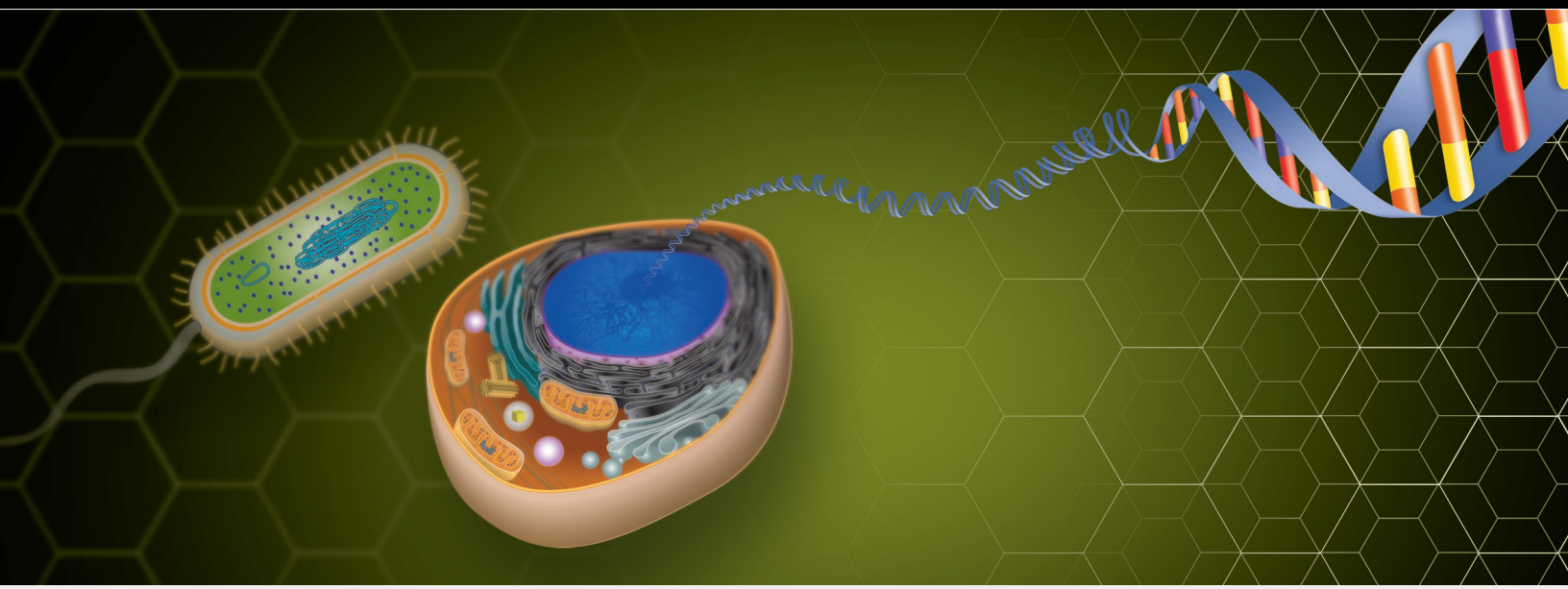
BIO-RAD Explorer



DNA Extraction Module

Catalog #12016408EDU

Instruction Manual

For technical support, call your local Bio-Rad office, or in the U.S. call **1-800-4BIORAD** (1-800-424-6723) option 2.

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Storage

When you receive the DNA Extraction Module:

1. Note storage location and record the batch numbers from the product labels.
2. Store the **DNA Extraction Module** at room temperature.

3. Visit **bio-rad.com/DNAExtractionMod** to download the most up-to-date instruction manual.

Technical Support is available at support@bio-rad.com or 1-800-4BIORAD, option 2.

Safety Guidelines

Eating, drinking, smoking, and applying cosmetics are not permitted in the work area. Wearing protective eyewear and gloves is strongly recommended. Students should wash their hands with soap before and after this exercise. If any solution gets into a student's eyes, flush with water for 15 min. Lab coats or other protective clothing should be worn to avoid staining clothes.

Always use proper safety precautions when collecting and processing samples. Some organisms are harmful and/or poisonous if handled improperly. Improper handling may result in serious injury or death. It is recommended that students work only with samples that have been positively identified as nontoxic, or otherwise use samples from a reliable retailer such as a grocery store, pet store, or science supply company. Use appropriate personal protective equipment minimally including gloves and safety goggles. Use extreme caution when using cutting utensils to prevent injury. Use standard microbiology safety practices when working with samples that may contain pathogenic bacteria. Place any materials that contacted bacteria in a 10% bleach solution for at least 20 min to decontaminate. Follow local regulations for further disposal recommendations.

Visit **explorer.bio-rad.com** to access Safety Data Sheets for individual products.

Components

The DNA Extraction Module includes materials for 16 samples, or 32 students working in pairs (one sample each) or groups of four (two samples each).

Materials included in this module

Item	Quantity
DNA Extraction Module	
Resuspension solution	5 ml
Lysis solution	5 ml
Neutralization solution	5 ml
Matrix	5 ml
Wash buffer	10 ml
Spin columns	20
2 ml capped microcentrifuge tubes	250

Required materials not included in this module

Item	Quantity
Equipment	
100–1,000 µl adjustable-volume micropipet and tips	8–16
Dry bath or water bath	1
Microcentrifuge, ≥14,000 x g	1
Inoculation loop, sterile, if using bacterial samples	16
Razor blade or plastic knife, new and unused, if using tissue samples	16
Weigh boat	16
Microcentrifuge tube rack	8–16
Permanent marking pen	8–16
Reagents and Consumables	
Bulk tissue samples or bacteria culture	1–16
Distilled water	3 ml
Ethanol, 95%	10 ml
Bleach solution, 10%	

Ordering Information

Catalog #	Description
Kits and Refill Packs	
12016408EDU	**DNA Extraction Module**
1665105EDU	**DNA Extraction Reagent Pack**
Reagents and Consumables	
2239430EDU	**EZ Micro Test Tubes**, 500
1660471EDU	**Inoculation Loops**, Sterile, 100
Equipment and Laboratory Supplies	
1660506EDU	**Professional Adjustable-Volume Micropipet**, 2–20 µl
1660507EDU	**Professional Adjustable-Volume Micropipet**, 20–200 µl
1660508EDU	**Professional Adjustable-Volume Micropipet**, 100–1,000 µl
1660562EDU	**Digital Dry Bath**, 120 V
1660504EDU	**Temperature-Controlled Water Bath**, 120 V
12011919EDU	**Mini Centrifuge**, 100–240 V
1660602EDU	**Model 16K Microcentrifuge**, 120 V
1660610EDU	**BR-2000 Vortexer**, 120 V
1660481EDU	**Green Racks**, set of 5

Visit **explorer.bio-rad.com** for a full list of 220–240 V equipment.

Preparation Instructions

1. Use Table 1 to prepare and dispense solutions up to four weeks before the activity.

To help prevent cross-contamination, label tubes and dispense one solution at a time. Be sure to use a new pipet tip for each solution.

Table 1. Reagent preparation and dispensing instructions.

Tube Type and Quantity	Tube Label	Tube Contents and Preparation Instructions	Storage
16 microcentrifuge tubes with caps, 2.0 ml	**R**	250 µl resuspension solution	Up to 4 weeks at room temp.
16 microcentrifuge tubes with caps, 2.0 ml	**Lys**	300 µl lysis solution	Up to 4 weeks at room temp.
16 microcentrifuge tubes with caps, 2.0 ml	**N**	300 µl neutralization solution	Up to 4 weeks at room temp.
16 microcentrifuge tubes with caps, 2.0 ml	**X**	250 µl resuspended matrix **Preparation:** Before dispensing, resuspend the resin matrix by shaking, stirring manually, or vortexing	Up to 4 weeks at room temp.
16 microcentrifuge tubes with caps, 2.0 ml	**Wash**	1 ml wash buffer plus ethanol solution **Preparation:** Before dispensing, add 10 ml of 95% ethanol to the bottle of wash buffer, replace the cap, and shake to mix.	1–2 days at room temp.
16 microcentrifuge tubes with caps, 2.0 ml	**dH2O**	150 µl distilled water	Indefinitely

2. Acquire tissue samples (if using) any time before the lessons.

Source tissue samples or have your student source tissue samples any time before the activity. To avoid spoilage and contamination, store tissue samples frozen and then thaw the day of the activity. Plant tissue samples are not recommended for this DNA extraction protocol.

Table 2. Samples, by species, that reliably produce successful results.

Robust			Less Robust	Difficult/Not Robust	
Insects					
Beetles Moths	Butterflies Bees	Wasps Flies	Spiders	Molted exoskeletons Cocoons	Gastropods
Mammals and Birds					
Cow Pig	Sheep Deer	Pheasant Duck	Elk Turkey	Ground meat Fur	Feathers Bones
Fungi					
Fresh mushroom caps			Dried mushrooms are less robust than fresh mushrooms, but can still produce good results	Older mushrooms, due to the risk of contamination	
Fish					
Dried fish Catfish Shark Sturgeon Trout Rock cod	True cod Tilapia Mackerel Yellowfin tuna Sea bass Opah	Imitation crab Flying fish roe Bonito flakes Shrimp	Salmon Salmon roe Anchovy Arctic char Sardine	Fried fish Canned fish Sea urchin Red tuna Mussel Clam	

3. Prepare bacteria cultures (if using) 1–5 days before the DNA extraction activity.

For each student sample, prepare a bacterial culture plate or 3 ml of liquid bacterial culture from a single colony. Chromosomal DNA can be extracted from either bacterial colonies or liquid culture.

4. Prepare student workstations.

Use Tables 3–4 to set up workstations just before the activity. Each workstation has enough materials to extract DNA from one sample. Micropipets can be shared if necessary between groups. Other materials should not be shared,to avoid contamination.

Table 3. Student workstation.

Materials	Quantity
Resuspension buffer (**R**)	250 µl
Lysis buffer (**Lys**)	300 µl
Neutralization buffer (**N**)	300 µl
Matrix (**X**)	250 µl
Wash buffer (**Wash**)	1 ml
Distilled water (**dH2O**)	250 µl
Empty 2 ml microcentrifuge tubes with caps	1
Empty 2 ml microcentrifuge tubes with caps removed	2
Spin column	1
Bulk tissue sample or bacterial culture	1
Empty, clean weigh boat	1
Inoculation loop, sterile, if using bacterial samples (**Note:** It is critical to use one loop per sample)	1
Razor blade, plastic knife, or other clean cutting utensil, if using tissue samples (**Note:** it is critical to use one utensil per sample)	1
100–1,000 µl adjustable-volume micropipet and tips	1
Marking pen	1
Bleach solution, 10%	

Table 4. Common workstation.

Materials	Quantity
Water bath or dry bath set to 55°C	1
Microcentrifuge	1–2

Tips and Notes

The success of many downstream analyses, including polymerase chain reaction (PCR), is highly dependent on the quality and quantity of DNA extracted from your samples. Use the following tips to ensure successul DNA extraction.

Preventing contamination is of utmost importance. Use the following precautions to minimize the chance of contamination.

- New, clean, and DNA-free cutting utensils are required for each sample. ***Do not*** cross-contaminate samples. The DNA from a single contaminating cell can ruin results. Use gloves and change them between specimens
- Wiping a utensil and rinsing in ethanol between samples ***will not*** sufficiently remove DNA present on a cutting utensil
- When working with non-bacterial samples, it is possible for DNA from contaminating bacteria to coamplify with sample DNA. Taking samples from the interior of bulk animal tissue can reduce the chances of contamination with bacteria
- When selecting samples, avoid fur, feathers, exoskeleton, bones, or other keratinous or chitinous samples. Also avoid canned or processed samples, as acidic conditions can damage DNA. High fat–content samples (for example, fried foods) may also inhibit DNA extraction and subsequent PCR. Fresh, frozen, or dried samples can produce excellent results
- Once lysis buffer is added during DNA extraction, there is no convenient stopping point until the DNA is eluted from the spin purification column. If you choose to stop before adding the lysis buffer, be sure student samples are labeled with their initials, and store them refrigerated at 4°C for up to 1 week
- Wipe down surfaces and rinse plastic pipet barrels, mortars, and pestles with 10% bleach to remove any surface DNA contamination.

Materials Required

Student Workstation

Materials	Quantity
Resuspension buffer (**R**)	250 µl
Lysis buffer (**Lys**)	300 µl
Neutralization buffer (**N**)	300 µl
Matrix (**X**)	250 µl
Wash buffer (**Wash**)	1 ml
Distilled water (**dH2O**)	150 µl
Empty 2 ml microcentrifuge tubes with caps	1
Empty 2 ml microcentrifuge tubes with caps removed	2
Spin column	1
Bulk tissue sample or bacterial culture	1
Empty, clean weigh boat	1
Inoculation loop, sterile, if using bacterial samples (**Note**: It is critical to use one loop per sample)	1
Razor blade, plastic knife, or other clean cutting utensil (**Note:** It is critical to use one utensil per sample)	1
100–1,000 µl adjustable-volume micropipet and tips	1
Marking pen	1
Bleach solution, 10%	

Common Workstation

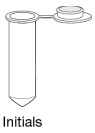
Materials	Quantity
Water bath or dry bath set to 55°C	1
Microcentrifuge	1–2

Protocol

1. **Label one capped 2 ml microcentrifuge tube with your initials.**

   If provided or known, record the ID or a description of the tissue sample you will be using.

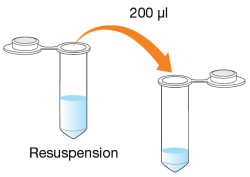
   Sample _____



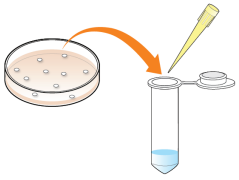
2. **Prepare your sample by following the steps below that correspond to your sample type.**

   For bacteria samples on solid media

   a. **Add 200 µl resuspension buffer (R) to the labeled 2 ml microcentrifuge tube.**



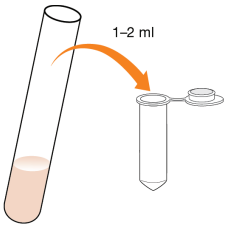
   b. **Use a new sterile pipet tip or inoculating loop to pick one bacterial colony from a culture plate and transfer to the tube.**



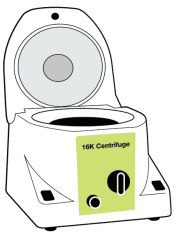
   c. **Completely resuspend the colony in the resuspension buffer by pipetting up and down or vortexing for 10–20 sec.**

   For bacteria samples in liquid culture

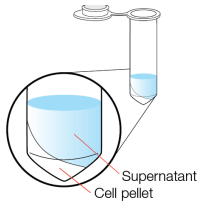
   a. **Transfer 1–2 ml fresh overnight bacteria liquid culture to the labeled 2 ml microcentrifuge tube.**



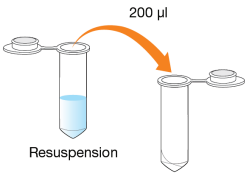
   b. **Centrifuge the sample at 14,000 x g for 30 sec to form a cell pellet. Be sure to use a counterbalance.**



c. **Use a micropipet to carefully remove the supernatant without disturbing the pellet. Discard the supernatant into a 10% bleach solution.**

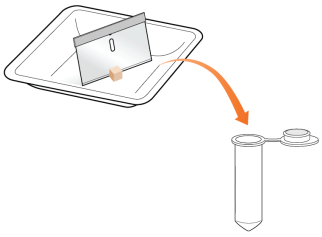


d. **Add 200 µl of resuspension buffer (R) and pipet up and down or vortex to completely resuspend the bacteria.**



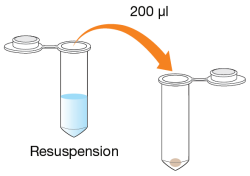
For fish, mammalian, bird, or fungal samples

a. **Cut a piece of tissue sample up to 100 mg, approximately the size of an eraser-head, from the bulk tissue sample and place it directly into the appropriately labeled 2 ml microcentrifuge tube.**



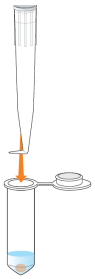
Note: Cut a sample from the interior of the bulk tissue to avoid bacterial contamination that may be present on the surface. Avoid fur, scales, or feathers.

b. **Add 200 µl resuspension buffer (R).**



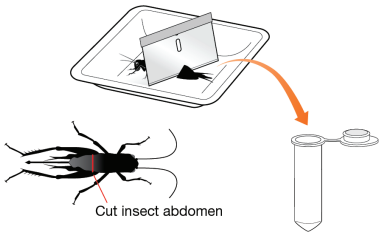
c. **Take a micropipet tip in hand and firmly press the narrow end at a slight angle into the sample weigh boat or a new weigh boat to bend and crush a small portion of the tip.**

d. **Use the crushed micropipet tip as a pestle to grind the sample in the microcentrifuge tube for at least 30 sec until the sample becomes cloudy. Then discard the tip.**



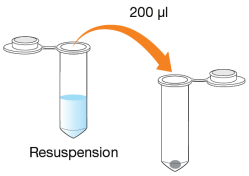
For insect samples

a. **Cut a small piece of tissue approximately half the size of an eraser-head from the abdomen of your insect. The total mass will vary. Place the piece directly into the appropriately labeled microcentrifuge tube.**



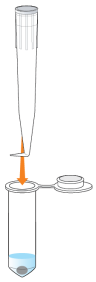
Note: Avoid wings, legs, and other chitinous parts of the insect body, as they are much less effective for DNA extraction.

b. **Add 200 µl resuspension buffer (R).**



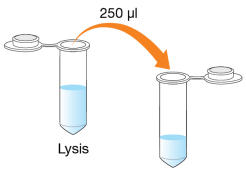
c. **Take a micropipet tip in hand and firmly press the narrow end at a slight angle into the sample weigh boat or a new weigh boat to bend and crush a small portion of the tip.**

d. **Use the crushed micropipet tip as a pestle to grind the sample in the microcentrifuge tube for at least 30 sec until the sample becomes cloudy. Then discard the tip.**

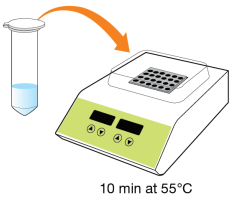


STOP **Stop. Ask your instructor whether to proceed now or later.**

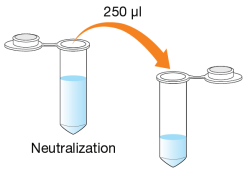
3. **Add 250 µl lysis buffer (Lys) and mix gently by inverting each tube 10 times. DO NOT VORTEX. Vortexing may shear genomic DNA, which can inhibit PCR amplification.**



4. **Incubate sample at 55°C for 10 min in a water bath or dry bath.**



5. **Add 250 µl neutralization buffer (N) and mix gently by inverting 10 times. DO NOT VORTEX. A cloudy precipitate may form.**



6. **Centrifuge for 5 min at 12,000–14,000 x g. Be sure to use a counter balance. A compact pellet will form along the side of the tube. The supernatant contains the DNA.**

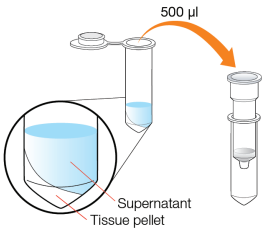
   If there are many particulates in the supernatant after centrifugation, centrifuge the tubes for an additional 5 min.

12,000–14,000 x g, 5 min

7. **Snap off (do not twist!) the bottom of the spin column. Insert the column into a capless 2 ml microcentrifuge tube.**

8. **Label the spin column with your initials.**

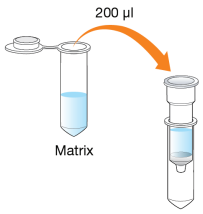
9. **Using a new pipet tip for each sample, transfer ~500 µl supernatant from each sample to the appropriately labeled spin column.**



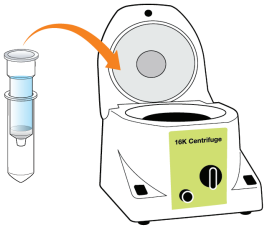
   Do not disturb the tissue pellet.

10. **Vortex or repeatedly shake the matrix (X) to make sure the resin is completely resuspended.**

11. **Quickly add 200 µl thoroughly resuspended matrix (X) to the spin column. Pipet up and down to mix.**



12. **Centrifuge the spin column with the capless microcentrifuge tube at full speed (14,000 x g) for 30 sec. Be sure to use a counter balance.**

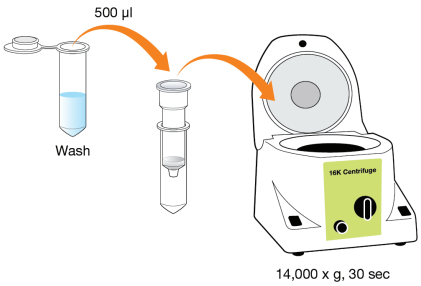


14,000 x g, 30 sec

   Centrifuge for only 30 sec! Completely drying the matrix at this point will result in DNA loss.

13. Remove the spin column from the capless 2 ml microcentrifuge tube, discard the flowthrough, and replace the spin column into the same tube.

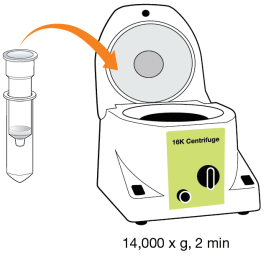
14. Add 500 µl wash buffer (**Wash**) and wash the matrix by centrifuging for 30 sec. Be sure to use a counterbalance.



⚠ **Centrifuge for only 30 sec! Completely drying the matrix at this point will result in DNA loss.**

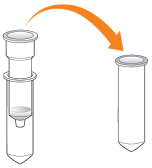
15. Remove the spin column from the capless 2 ml microcentrifuge tube, discard the flowthrough, and replace the spin column into the same tube.

16. Finally, centrifuge the spin column with the capless microcentrifuge tube at full speed for a full 2 min to remove residual traces of ethanol and dry out the matrix. Be sure to use a counterbalance.

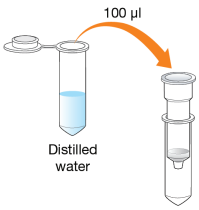


17. Label a clean 2 ml capless microcentrifuge tube with your initials.

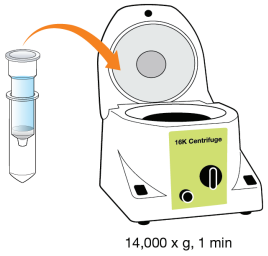
18. Transfer the spin column into the appropriately labeled new, clean capless microcentrifuge tube. Discard the used capless microcentrifuge tube along with any liquid in the tube.



19. Using a new pipet tip, add 100 µl distilled water to the spin column, being careful not to touch the resin.

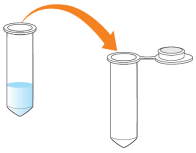


20. Centrifuge the spin column with the capless microcentrifuge tube at full speed (14,000 x g) for 1 min. Be sure to use a counterbalance.



21. Label a new 2 ml capped microcentrifuge tube with your initials.

22. Remove the spin column from the capless microcentrifuge tube and transfer the flowthrough which contains eluted DNA, into the appropriately labeled 2 ml capped microcentrifuge tube.



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BIO-RAD

Bio-Rad Laboratories, Inc.

Life Science Group

Website *bio-rad.com* ***USA*** *1 800 424 6723* ***Australia*** *61 2 9914 2800* ***Austria*** *00 800 00 24 67 23* ***Belgium*** *00 800 00 24 67 23* ***Brazil*** *4003 0399*
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