

ATCC15834 Agrobacterium tumefaciens [pRi15834]

TECHNICAL MANUAL -- SPECIFICATION & PROTOCOLS

PRODUCT TECHNICAL PROFILE

Catalog Number:	S0070
Strain Name:	Agrobacterium tumefaciens (ATCC15834)
Taxonomic Classification:	Agrobacterium rhizogenes (Rhizobiaceae family)
Host Organism:	Gram-negative soil bacterium
Genotype / Plasmid:	pRi15834 (agropine-type Ri plasmid)
Resistance(s):	Kanamycin (Kan), Spectinomycin (Spec)
Culture Medium:	YEB / YMB / TY
Growth Conditions:	Aerobic, 28C
Storage:	Glycerol stock, -80C
Form:	Glycerol stock (100 uL)
Biosafety Level:	BSL-1
Shipping Temperature:	2--8C
Source:	ATCC (American Type Culture Collection)

COMPLETE GENOTYPE / PLASMID PROFILE

Agrobacterium rhizogenes pRi15834 (agropine-type Ri plasmid)

STRAIN DESCRIPTION

Taxonomy & Classification: Agrobacterium tumefaciens ATCC15834 is a Gram-negative soil bacterium belonging to the genus Agrobacterium in the Rhizobiaceae family. It is reclassified as Agrobacterium rhizogenes based on its root-inducing (Ri) plasmid characteristics.

Ri Plasmid Type: pRi15834 agropine-type Ri plasmid -- transfers T-DNA from the right border (RB) region into plant genomes, inducing hormone-independent hairy root formation.

Culture Medium: YEB (Yeast Extract Broth), YMB (Yeast Extract-Mannitol Broth), or TY (Tryptone-Yeast extract medium)

Growth Conditions: Aerobic atmosphere at 28C. Optimal growth occurs within 48--72 hours on solid media.

Antibiotic Resistance: Kanamycin (130 ug/mL working concentration); Spectinomycin (70 ug/mL working concentration)

ATCC15834 is a wild-type strain derived from the American Type Culture Collection (ATCC). It carries the pRi15834 agropine-type Ri plasmid with a broad host range capable of infecting most dicotyledonous plants, several monocotyledonous plants, and some gymnosperms. The strain is particularly well-suited for inducing hairy roots in medicinal plants such as paclitaxel-producing species (Taxus spp.) and Artemisia annua (artemisinin source), as well as model plants including tobacco, legumes (Fabaceae), and grasses (Poaceae).

The transformed hairy root cultures exhibit rapid growth without exogenous hormones, genetic stability of the integrated T-DNA, and elevated biosynthesis of secondary metabolites compared to non-transformed root cultures or callus systems.

Bacterial strain. Genotype: Agrobacterium rhizogenes pRi15834 (agropine-type)

Host Range: Poaceae, Fabaceae, Solanaceae (tobacco), gymnosperms, medicinal plants (paclitaxel, artemisinin)

Atmospheric Requirements: Aerobic, optimal temperature 28C

ANTIBIOTIC SELECTION & WORKING CONCENTRATIONS

Antibiotic	Stock Solution	Working Concentration	Screening Medium	Preparation Method
Kanamycin (Kan)	100 mg/mL	130 ug/mL	YMB or TY	Dissolve in ddH2O, filter sterilize through 0.22 um membrane
Spectinomycin (Spec)	50 mg/mL	70 ug/mL	TY	Dissolve in ddH2O, filter sterilize through 0.22 um membrane

CULTURE MEDIA FORMULATIONS

YMB Medium (Yeast Extract-Mannitol Broth) -- Formula 0217 (per Liter)

Component	Amount
Yeast Extract	1.0 g
MgSO4·7H2O	0.2 g
K2HPO4	0.5 g
NaCl	0.1 g
Mannitol	10.0 g
Distilled Water	to 1000 mL
pH (adjusted)	7.0 +/- 0.2
Sterilization	121C for 20 minutes (autoclave)
For solid medium	Add 15 g/L agar powder before autoclaving

TY Medium (Tryptone-Yeast Extract) -- per Liter

Component	Amount
Tryptone	5.0 g
Yeast Extract	3.0 g
Distilled Water	to 1000 mL
Sterilization	121C for 20 minutes (autoclave)
Calcium Chloride Supplement	Add 10 mL sterile 1M CaCl2 solution per liter after autoclaving
CaCl2 Stock Solution	Prepare 1M aqueous solution, autoclave separately at 121C for 20 min
For solid medium	Add 15 g/L agar powder before autoclaving

STRAIN RECOVERY & CULTURE PROTOCOL

1. **Prepare Culture Medium** Prepare YEB, YMB, or TY agar plates (or slants) according to the formulations above. Pre-warm plates to room temperature before inoculation.

Note: For antibiotic selection, add appropriate antibiotics (Kan 130 ug/mL or Spec 70 ug/mL) to cooled molten medium (~50C) before pouring plates.

2. **Thaw Glycerol Stock** Remove cryovial from -80C storage and thaw on ice or at room temperature. If already melted upon receipt, proceed immediately to step 3.

Note: This product ships at 2--8C. Upon receipt, transfer immediately to -80C if not culturing right away.

3. **Surface Disinfection** Wipe the exterior of the cryovial thoroughly with a 75% ethanol swab before opening inside a laminar flow hood.

Note: Allow ethanol to evaporate completely before opening the cap to prevent contamination of the glycerol stock.

4. **Inoculation** Open the cap under sterile conditions. Using a sterile inoculation loop or pipette, transfer an appropriate amount of bacterial suspension onto the prepared plate/streak surface.

Note: Use the four-quadrant streak method on plates to obtain isolated single colonies.

5. **Incubation** Place plates upside down (agar side up) in an incubator set to 28°C under aerobic conditions.

Note: **ATCC15834 grows slowly.** On antibiotic-containing plates, transformed bacteria require up to 72 hours (3 days) of incubation for visible colony formation. On non-selective media, colonies typically appear within 48–72 hours.

6. **Colony Selection & Expansion** After incubation, select well-isolated single colonies of appropriate morphology and subculture onto fresh medium as needed for downstream applications.

Note: Verify colony purity by confirming uniform morphology before large-scale culture expansion.

HAIRY ROOT INDUCTION -- APPLICATION GUIDELINES

ATCC15834 carrying the pRi15834 agropine-type Ri plasmid is specifically optimized for the following applications:

- **Medicinal Plant Hairy Root Cultures:** Paclitaxel (Taxol) production from *Taxus* species; Artemisinin production from *Artemisia annua*; Ginsenoside production from *Panax* species
- **Model Plant Systems:** Tobacco (*Nicotiana tabacum*), *Arabidopsis thaliana*, carrot (*Daucus carota*) disk transformation assays
- **Crop Species:** Legumes (Fabaceae), grasses (Poaceae), Solanaceous vegetables

Key Transformation Parameters:

- Co-cultivation temperature: 22–25°C (lower than standard growth temperature improves T-DNA transfer efficiency)
- Acetosyringone supplementation: 100–200 µM in co-cultivation medium enhances virulence gene induction
- Infection duration: 24–72 hours depending on plant tissue type
- Post-infection selection: Apply appropriate antibiotic (Kan/Spec) to select for transformed roots

Caution:

1. This product is FOR RESEARCH USE ONLY!
2. This product is glycerol packed. Please culture it as soon as possible after receipt, then harvest and store at -80°C.
3. Shipping temperature is 2–8°C. Store long-term at -80°C.
4. ATCC15834 grows slowly. Transformed bacteria on antibiotic-containing plates require up to 72 hours for colony formation. Do not discard plates prematurely.
5. Please use the active culture as soon as possible; active cultures are not recommended for long-term storage.
6. If other culture mediums or growth conditions are used, our warranty for viability is no longer valid.
7. Users should store, handle, and dispose of the strains under safe and appropriate conditions.

Important Handling Notes

1. This product is classified as **BSL-1**. Standard microbiological practices and aseptic technique are sufficient for safe handling.
2. Although BSL-1, this strain contains a conjugative Ri plasmid capable of horizontal gene transfer. Avoid cross-contamination with other bacterial cultures in the same workspace.
3. All waste materials must be decontaminated by autoclaving before disposal. Disinfect work surfaces with 70% ethanol or appropriate disinfectant after use.

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For research use only. Not for diagnostic or therapeutic applications.