



PathoID Inc.

*Assays from Imagination to Reality*

## Amelogenin PCR Kit

Education and Hobbyist Series

p/n: 020020101

Application: Amplification of Amelogenin loci from extracted DNA samples (e.g. buccal cell DNA extracts, see PathoID kit 020010101).

Kit size: 25 samples.

Storage conditions: -20°C. Product stability has not been extensively tested, and no expiration date is specified as of this product revision. Stability under these conditions expected to be > 6 months.

### Contents:

- 1 x 2.0 ml, PCR grade water (clear cap) – *tested free of detectable DNase or amplifiable human DNA*
- 1 x 55 µl, Amelogenin primer mix (yellow caps)
- 1 x 345 µl, Polymerase Mix (red cap)
- 1 x 12 µl, Control Template – *Positive control human male DNA, 50 – 100 pg/µl. Enough for 12 control reactions.*

As needed, use a microcentrifuge to collect any reagents at bottom of tube before use.

### User Equipment Required:

- Micropipettors and pipette tips (1 ml, 200 µl, and 10 µl sizes suggested)
- PCR thermocycler
- PCR tubes compatible with user thermocycler
- Agarose gel system, gel loading dye, size ladder, power supply
- Transilluminator
- Camera – *Optional; if results record desired*

Protocol:

1. Wear appropriate PPE and take standard laboratory precautions when using this kit.
2. Avoid contamination of the sample or collection tubes with exogenous DNA as much as practical for your setting.
3. For each sample to be processed, get and label one PCR Tube. Label two more, one as Positive Control and one as Negative Control.
4. Thaw out Amelogenin PCR Kit contents.
5. Prepare a polymerase **Master Mix** for each sample to be tested, plus one Positive Control and one Negative Control. Per total number of reactions, mix the following in an additional, unlabelled PCR Tube:

<i>Component</i>	<i>Volume/rxn</i>	<i>e.g 3 samples + controls</i>
PCR Water (clear cap)	10.5 µl	52.5 µl
Amelogenin Primer Mix (yellow cap)	2.2 µl	11 µl
Polymerase Mix (red cap)	13.75 µl	68.8 µl

6. Mix gently by pipetting, then dispense 19 µl Master Mix to each labelled PCR tube.
7. Add 1 µl of sample template to each matching labelled PCR tube. To the Positive Control PCR tube, add 1 µl Control Template (blue cap); to the Negative Control PCR tube, add 1 µl PCR Water (clear cap).
8. *OPTIONAL – If user PCR machine requires addition of mineral oil to samples, i.e. it does not have a heated lid, add mineral oil on top of all samples and controls now.*
9. Close caps.
10. In user's PCR Thermocycler, cycle as:
  - a. 95°C – 2 minute
  - b. 40 cycles of:
    - i. 95°C – 20 seconds
    - ii. 56°C – 30 seconds
    - iii. 72°C – 1 minute
  - c. 20°C – 1 minute
  - d. end
11. Analyze 5 µl portions of the PCR products for each sample by electrophoresis on 2% agarose gel, next to a size standard. Use of SB buffer is recommended for optimal resolution.
12. Negative control should show no bands at 106 or 112 bp (*faint lower size smearing from primer dimer artifacts is sometimes seen and is acceptable*).
13. Positive Control should show a doublet of bands as 106 and 112 bp. *If these are hard to discern, you may need to run the gel longer.*

**Reading the results:**

This test will generate a single band at 112 bp size when human Y chromosomes are present in a sample, and a single band at 106 bp when human X chromosomes are present in a sample. Thus, a normal male sample will generate a close but resolvable 106/112 doublet band, and female samples will generate a single 106 bp band. Bands not corresponding to these sizes are amplification artefacts and can be ignored. Unexpected bands corresponding to the correct sizes are indicative of sample contamination or subject aneuploidy.

**Marker background:**

This marker tests for a section of the Amelogenin gene, which is essential in the proper formation of tooth enamel. The gene is unusual in that copies are carried on both sex chromosomes (AMELX on X and AMELY Y), with the Y chromosome locus having a 6 base pair insertion in its DNA sequence relative to the X chromosome locus. This test detects and sizes a region of the gene around this size difference, allowing identification of male from female derived samples. This marker is frequently used as a sample sex determination marker in DNA forensics and anthropology applications.

## References:

- 1: <http://ghr.nlm.nih.gov/gene/AMELX>
- 2: Detection of the CCR5-Delta32 HIV resistance gene in Bronze Age skeletons. Hummel S, Schmidt D, Kremeyer B, Herrmann B, Oppermann M. Genes Immun. 2005 Jun;6(4):371-4.

Quality Control

Each lot of this kit is functionally tested to perform as expected when following the above protocol on control material as included.

**CAUTIONS AND DISCLAIMERS:** This kit is provided for **EDUCATIONAL AND ENTERTAINMENT USE ONLY**. Use in any medical diagnostic, clinical, forensic, or agricultural application is not approved, and by use of this product, you agree no such applications will be made. In the event any potentially medically relevant results are suggested through use of this product, the user is recommended to discuss the matter with a qualified medical specialist.

This kit is intended for use by or under the supervision of adults with an understanding of, and in accordance with, basic laboratory safety procedures including but not limited to personal protective equipment (PPE).

**KIT CONTENTS** non-toxic. All kit products and wastes are non-biohazardous and may be disposed of in routine domestic waste streams in most jurisdictions. If in doubt, please consult local regulations.

PathoID does not warranty this product for any specific end user application other than its intended use when instructions are followed. PathoID accepts no liability, direct or indirect, for the use of this product. In the event of perceived product failure, the end user is recommended to contact PathoID Customer Service at (503) 951-4299 or [custserv@pathoid.com](mailto:custserv@pathoid.com) with product lot number and description of problem. Sole remedy in the event of a product failure shall be, at PathoID's discretion, either refund for or replacement of the non-performing portion(s) of the product.

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