

**University Children's Genetics Laboratory, A Division of ProGene, Inc.
Molecular Genetics Lab**

PHENYLKETONURIA 15 POINT MUTATION & LINKAGE

SYNONYMS: PKU, Phenylalanine Hydroxylase (PAH) Deficiency; Folling Disease.

CPT: 83891 (molecular isolation & extraction); 83892 (enzymatic digestion); 83893 (dot blot); 83894 (separation); 83896 (nucleic acid probe); 83898 (amplification single); 83900 (amplification duplex), 83901 (amplification multiplex); 83912 (interpretation & report)

TEST INCLUDES: Point mutation detection; Linkage Analysis.

LAB: University Children's Genetics Laboratory/ Molecular Section

PHONE: (818) 548-0999

REQUEST FORM: Molecular lab requisition & the signed consent form

TURN AROUND TIME: 2-3 Weeks (after at least 3 patients have requested the test)

PRICE: *PKU-mutation panel analysis:* *** \$650.00
PKU-direct mutation (screening for 2 known mutations): \$300.00
Prenatal diagnosis (2 known mutations + maternal contamination study): \$500.00
Linkage analysis: \$350.00 per person or \$900.00 per family up to 6 individuals

SPECIMEN: *Whole blood:* 8-10 ml (minimum: 5 ml); Purple top (EDTA) or Yellow top (ACD) tube
Store specimen refrigerated; may be stable up to 7 days.
Specimen may be rejected if hemolyzed, clotted, or improperly stored.
Amniotic fluid: 4 T-25 flasks of cultured cells
Chorionic villi: 4-T-25 flasks of cultured cells

INDICATION FOR TESTING:

Patients with clinical indication or family history; Carrier screening in specific populations;
Prenatal diagnosis with documented familial mutation(s)

LIMITATIONS: Mutations in PKU patients are variable over the world. With the 15 mutations offered at our laboratory, the detection rate is approximately 50%.

METHOD: **Point mutation** analysis uses both PCR/RFLP and PCR/ASO hybridization assays to identify 15 common mutations.

*** **NOTE:** *Due to efficiency reasons, the PKU mutation panel is currently offered only for 3 or more patients.*

Linkage analysis is available when mutation analysis fails to detect both PKU alleles. Short Tandem Repeats (STR) in intron 3, Variable Number of Tandem Repeats (VNTR) 3' of exon 13, and *Xmnl* Restriction Fragment Length Polymorphism (RFLP) in intron 8 are used to trace the transmission of mutant *PAH* gene(s) in informative PKU families with one or more affected children. Linkage analysis is used for prenatal diagnosis and carrier testing with confirmed family history of PKU.

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ADDITIONAL INFO:

PKU, an autosomal recessive disease, is the most common inborn error of amino acid metabolism. The prevalence of hyperphenylalaninemia (HPA) in Caucasian and East Asian populations is one in 10,000 live births. The lack of phenylalanine hydroxylase (PAH) activity causes persistent hyperphenylalaninemia, with irreversible impairment of brain development resulting in severe mental retardation in untreated children. Some forms of the disease are caused by disorders of synthesis and recycling of tetrahydrobiopterin (BH₄), the cofactor, involved in the phenylalanine hydroxylation reaction. BH₄ deficiency accounts for approximately 2% of patients with HPA.

The PAH gene, located on chromosome 12q22-24, consists of 13 exons and spans 90 kilobases. The gene product is a cytosolic enzyme expressed in liver. Our laboratory tests for the 15 most common mutations seen in the Caucasian population, including: E280K, P281L, R408W, IVS10nt-11g>a, IVS12nt1g>a, R243X, F39L, L48S, I65T, R158Q, V245A, R261Q, A403V, R408Q, Y414C. The limitation is that the detection rate is about 50% among affected patients.

REFERENCES:

1. Goltsov A. et al, Hum Mol Genet (1993) 2:577-581
2. Lidsky A. et al, Am J Hum Genet (1985) 37:619-634
3. Scriver CR, Kaufman S, Eisensmith E, Woo SLC, Vogelstein B, Childs B (eds) The Metabolic and Molecular Bases of Inherited Disease, 8 ed. McGraw Hill, NY, Ch 78

RESOURCES:

1. Gene Clinics
<http://www.geneclinics.org/profiles/pku/index.html>
2. National PKU News
<http://www.pkunews.org>
3. National Society of PKU
web.ukonline.co.uk/nspku
4. OMIM 261600
<http://www.ncbi.nlm.nih.gov/htbin-post/Omim/dispmim?261600>
5. PAH/PKU Resource Booklet for Families
<http://www.pahdb.mcgill.ca/?Topic=Information&Section=Clinical&Page=1>
6. Phenylalanine Hydroxylase Locus Knowledgebase
<http://www.pahdb.mcgill.ca>
7. Tetrahydrobiopterin Home Page
<http://www.bh4.org/>

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PHENYLKETONURIA SEQUENCING

SYNONYMS: PKU, Phenylalanine Hydroxylase (PAH) Deficiency; Folling Disease.

CPT: 83891 (molecular isolation & extraction); 83892 (enzymatic digestion); 83893 (dot blot); 83894 (separation); 83896 (Probe); 83904 (mutation identification by sequencing, each segment); 83898 (amplification single), 83912 (interpretation & report)

TEST INCLUDES: Sequencing of up to 13 exons

LAB: University Children's Genetics Laboratory/ Molecular Section

PHONE: (818) 548-0999

REQUEST FORM: Molecular lab requisition & the signed consent form

TURN AROUND TIME: 3-4 months

PRICE:	<i>Patient (proband) direct mutation analysis by sequencing.</i>	\$1,800.00
	<i>Carrier test</i> for at risk family member once mutation in proband is found:	\$300.00
	<i>Prenatal test</i> , with known mutations:	\$500.00

SPECIMEN: **Whole blood from patient:** 8-10 ml (minimum: 5 ml);
Purple top (EDTA) or Yellow top (ACD) tube
Whole blood from biological parents, for mutation confirmation
(however, results are not reported)
Store specimen refrigerated; may be stable up to 7 days.
Specimen may be rejected if hemolyzed, clotted, or improperly stored.
Amniotic fluid: 4 T-25 flasks of cultured cells
Chorionic villi: 4-T-25 flasks of cultured cells

INDICATION FOR TESTING:

Patients with clinical indication or family history; prenatal diagnosis

METHOD: PCR and sequencing primers are designed according to published data. A second molecular testing method, such as RFLP or ASO, is employed to confirm positive sequencing results.

We request parental bloods be submitted along with patient blood to confirm mutations.
(However, results are not released or reported for parents, without a separate requisition.)

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The PAH gene, located on chromosome 12q22-24, consists of 13 exons and spans 90 kilobases. The gene product is a cytosolic enzyme expressed in liver. Today, there are about 400 PKU mutations reported. Our laboratory uses PCR-based sequencing technology to identify mutations among the 13 exons. This method identifies approximately 99% of mutations, but since it is PCR-based, it will not detect deletions or rearrangements (if one normal allele is present, its amplification will obscure the mutant allele). Once the mutations are found, we use a second method such as RFLP or ASO, to confirm the mutations in the affected patient. If blood specimens are available from the biological parents, we confirm that the mutations identified are *in cis*. (Please note: parental results are not reported without a separate requisition and charge.)

REFERENCES:

1. Scriver CR, Kaufman S, Eisensmith E, Woo SLC, Vogelstein B, Childs B (eds) (2001) *The Metabolic and Molecular Bases of Inherited Disease*, 8 ed. McGraw Hill, NY, Ch 78

RESOURCES:

1. Gene Clinics - <http://www.geneclinics.org/profiles/pku/index.html>
2. National PKU News - www.pkunews.org
3. National Society of PKU - web.ukonline.co.uk/nspku
4. OMIM 261600 - <http://www.ncbi.nlm.nih.gov/htbin-post/Omim/dispmim?261600>
5. PAH/PKU Resource Booklet for Families - <http://www.pahdb.mcgill.ca/?Topic=Information&Section=Clinical&Page=1>
6. Phenylalanine Hydroxylase Locus Knowledgebase - <http://www.pahdb.mcgill.ca>
7. Tetrahydrobiopterin Home Page - <http://www.bh4.org/>

Order PKU-Panel to have the *PHA* gene screened for 15 point mutations (\$650). Order PKU-Seq for full mutation detection (\$1800). **If PKU-SEQ is ordered and two mutations are identified from the PKU-Panel*, only \$650 will be charged.**

(*Due to efficiency issues, the 15 point mutation panel is only performed when it is ordered for three or more patients. Please indicate when ordering PKU-Seq whether you prefer to wait for us to batch your test with others to perform the more economical PKU-Panel first. There is *no additional charge* (i.e. normal price of \$1800) for subsequent sequencing if the sample is negative for mutations in the panel.) Revised 12/18/03