

## Assay Summary

### MECP2 Gene Mutation Analysis

#### Rett Syndrome, infantile encephalitis, autism, and X-linked mental retardation

##### *Synopsis*

Rett syndrome is a X-linked dominant, progressive neurological disorder that primarily affects females, due to the lethality of severe mutations in males. It is characterized by normal development early on in life, followed by an arrest in development, and subsequently, a regression in language and motor skills. Most individuals typically develop loss of purposeful hand movements, and instead develop stereotypical repetitive hand motions. Approximately 50% of these individuals will also develop seizures. Severe mutations in the MECP2 gene have been associated with “classic” Rett syndrome. Mutations in MECP2 have also been observed in patients with “atypical” Rett syndrome, which is considered to be a milder form of this disease due to “mild” mutations in the MECP2 gene. Clinical features include mental retardation, mild learning disabilities and/or autism. The syndrome shows X-linked dominant inheritance and complete penetrance but is notable for some variability in the severity of clinical manifestations. Defects in the gene that encodes MECP2 (the Methyl-CpG-Binding Protein 2), impair gene transcription. The MECP2 protein functions by binding, and thereby silencing, genes that are controlled via methylation of CpG islands.

In addition, there is evidence that other milder mutations in MECP2 predispose to autism and X-linked mental retardation, although more work is necessary to confirm these findings and to estimate the frequency at which MECP2 contributes to these disorders.

##### *Indications for testing*

- 1) Individuals with a clear diagnosis of Rett syndrome, neonatal encephalitis, autism, or X-linked mental retardation, with or without a family history of the disease.
- 2) Individuals with a suspected diagnosis of Rett syndrome, neonatal encephalitis, autism, or X-linked mental retardation. Once a specific mutation is known in a family, presymptomatic testing for appropriate family members can be performed.

##### *Methodology*

**MECP2 sequence analysis.** All exons and associated intron junctions of the MECP2 (exons 1-4) are analyzed by direct DNA sequence analysis using an automated fluorescent sequencer. When a mutation is detected, confirmation is carried out by sequencing in the opposite direction, in a second independent PCR amplification. If no mutation is found, sequence analysis is performed in both directions. At-risk family members can be offered DNA sequence analysis of only the region of the gene with the previously identified mutation.

**MECP2 MLPA analysis:** We have incorporated the SALSA MLPA (multiplex ligation-dependent probe amplification) kit that is a rapid, high throughput technique for copy number quantification, specifically testing for large deletions/duplications for the exons 1-4 of the MECP2 gene. This assay should be considered for patients where full gene sequencing did not detect a mutation in the MECP2 gene.

### ***Performance/Limitations***

Sequencing analysis in patients with classic Rett syndrome detects mutations in 70-90% of individuals. Large deletion analysis identifies an additional 10-20% of the causal mutations. For mutation analysis, these methods will not detect mutations located in regions of the gene that are not analyzed (intron sequences other than the splice junctions, and upstream and downstream sequences). Some sequence alterations that may be detected (such as those causing missense or synonymous changes) will be of unknown clinical significance. As mutations and/or polymorphisms very close to the probe ligation site may result in a reduced relative peak area, apparent deletions/duplications detected by a single MLPA probe always require confirmation by other methods. Interpretation of test results should be in the context of the patient's ethnicity, clinical and family histories, and other laboratory test results.

### ***Specimen Requirements***

(a) Blood samples: 2 tubes with a total of 6 ccs in ACD (yellow top) or EDTA (lavender top) tubes. Keep at ambient temperature and ship by overnight courier. Samples must be received in our laboratory within 72 hours of draw.

**Note:**

- i) for infants, a minimum of 3 ccs is sufficient.
- ii) we accept DNA; at least 10 micrograms is required.

(b) Prenatal samples: 2 T25 flasks of confluent cells sent padded to arrive on M/Tu/W. A blood sample from the mother may be required (2 tubes with a total of 6 ccs in ACD (yellow top) or EDTA (lavender top) tubes) for use as positive control. Maternal cell contamination studies are not done here but are required for autosomal disorders and dosage analysis of X-linked disorders. We would be happy to assist in coordinating sending out a specimen for this purpose.

### ***Test Request Form (TRF)***

A completed MDL [TRF](#) is required for each specimen. Please submit the completed TRF with the specimen. Complete testing and billing information must be provided before the specimen is processed.

<b><i>Order Codes</i></b>	<b><i>CPT Codes</i></b>	<b><i>TAT</i></b>
MECP2-SEQ (MECP2 gene, full gene sequencing)	83890, 83898(x6), 83894, 83904(x6), 83912	5 wks
MECP2-CAS (MECP2 gene, targeted mutation analysis, known mutation)	83890, 83898, 83894, 83904, 83912	3 wks
MECP2-PD (MECP2 gene, known mutation detection, prenatal)	83890, 83898, 83894, 83904, 83912	2 wks
MECP2-DEL (MECP2 gene, MLPA analysis)	83890, 83896 (x13), 83909, 83912	3 wks
MECP2-DEL-CAS (MECP2 gene, MLPA analysis, known deletions/duplications)	83890, 83896 (x13), 83909, 83912	3 wks
MECP2-DEL-PD (MECP2 gene, MLPA analysis, prenatal)	83890, 83896 (x13), 83909, 83912	3 wks

### ***References***

1. Amir, R.E. et al. (1999) Nat. Genet. 23:185-188
2. Couvert, P. et al. (2001) Hum. Molec. Genet. 10:941-946
3. Lam, C. W. et al. (2000) J. Med. Genet. 37:E41

NOTE: This test is performed pursuant to a license agreement with Roche Molecular Systems, Inc.