

## NOD.Cg-Prkdc<sup>scid</sup>/J

Stock No: 001303

Protocol 19801: End Point Analysis Assay - Prkdc&lt;scid&gt; End Point

Version 4.0

### Notes

The genotyping protocol(s) presented here have been optimized for reagents and conditions used by The Jackson Laboratory (JAX). To genotype animals, JAX recommends researchers validate the assay independently upon receipt of animals into their facility. Reaction cycling temperature and times may require additional optimization based on the specific genotyping reagents used.

### Expected Results

Mutant = TAA

Wild type = TAT

amplicon = 149 bp

### Sequence

```

CTGTTCCAGTTATAGATCTTTGTTTTAGGGTCATTACTTGGTTTAAATGTT
TTTTAATGTAATTTGTATATGCTATTATAATAAGTAGAAAAAATGTGTT
TTTTCCCTTAGAGTTTTGAGCAGACAATGCTGAGAAAAGGAGGATCATGG
ATTCAAGAAATAAATGTAACGGAAAAGAATTGGTATCCACAACATAAAAT
ACGCTA[t/a]GCTAAGAGAAAAGTTAGCAGGGGCCAACCCAGCTGTTATAACTT
GGTAAGACTTGTGAATGCAGAATCAGTGTGTGTTCAAAAGTGCAAAGCAC
TTCACACACTTCTGAGCAGTATGGCACTTCACTGTGTAGATGGAGAAAGT
GACTCTTAGGGGGCTTTACCCCTCCAAGCCAGCCTGCAAGGACTGGGC
TCACACCCCTTGTC
  
```

### JAX Protocol

#### Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
14102		CAG ACA ATG CTG AGA AAA GGA G		Forward	A	
14103		CTG CAT TCA CAA GTC TTA CCA AG		Reverse	A	
14104	Fluorophore-1	TAA AAT ACG CTA AGC TAA GAG AAA G	Quencher-1	MUT Probe		
14105	Fluorophore-2	TAA AAT ACG CTA TGC TAA GAG AAA G	Quencher-2	WT Probe		

#### Reaction A

COMPONENT	FINAL CONCENTRATION
Kapa Probe Fast QPCR	1.00 X
ddH2O	
14102	0.40 uM
14103	0.40 uM
Wt Probe	0.15 uM
Mutant Probe	0.15 uM
DNA	

#### Cycling

STEP	TEMP °C	TIME	NOTE
1	95.0	--	
2	95.0	--	
3	60.0	--	
4		--	repeat steps 2-3 for 40 cycles
5	4.0	--	Forever

JAX uses a very high speed Taq (~1000 bp/sec), use cycling times recommended for your reagents.

Endpoint Fluorescence Scatter Plot

