

## **XBP1 Splicing Assay**

Perform PCR using cDNA from UPR induced cells.

### **XBP1 PCR (using Qiagen HotStar Taq #203203)**

	<b>1X</b>	<b>12X</b>
cDNA	2 $\mu$ L	-
10X PCR buffer	5 $\mu$ L	60
10mM dNTPs	1 $\mu$ L	12
HotStar Taq	0.25 $\mu$ L	3.0
5Q	10 $\mu$ L	120
25mM MgCl <sub>2</sub>	1 $\mu$ L	12
primer 1	1 $\mu$ L	12
primer 2	1 $\mu$ L	12
H <sub>2</sub> O	28.75 $\mu$ L	345
Total	50 $\mu$ L	48each

### **XBP1 PCR (using BioRad iTaq #170-8875)**

	<b>1X</b>	<b>12X</b>
cDNA	2	-
10X PCR buffer	5	60
50mM MgCl <sub>2</sub>	1.5	18
10mM dNTPs	1	12
iTaq	0.25	3
primer 1	1	12
primer 2	1	12
water	38.25	459
total	50	48each

### **Cycling Parameters**

Denature	95°C	15m	} 30 cycles
Denature	95°C	0.5m	
Anneal	55°C	0.5m	
Elongate	72°C	1m	
Final	72°C	10m	

Depending on primers, 10 $\mu$ L of PCR can be ran on a 1.5% agarose gel in 0.5X TBE.

OR...

Take 5 $\mu$ L of PCR reaction, and 10 $\mu$ L of sample buffer for acrylamide gel electrophoresis.

### **13% Native Acrylamide Gel**

#### **13% acrylamide stock solution**

40% acrylamide/bis 19:1	33.3mL
5X TBE	20mL
ddH <sub>2</sub> O	to 100mL

Take 25mL of 13% acrylamide stock, add 13 $\mu$ L TEMED, 110 $\mu$ L 10%APS, and pour gel. Run gel at 15mA.