The AQ-28 is a questionnaire measuring autistic traits, that is, traits that are related to Autism Spectrum Conditions, but its reliability in other cultures has not been thoroughly evaluated. This study tested whether the properties of the AQ-28 are comparable between two countries with different cultures, Malaysia and the Netherlands. A total of 437 Malaysian and 818 Dutch participants completed the AQ-28 online. The study measured whether the AQ-28 measures autistic traits similarly in Malaysia and the Netherlands. The AQ-28 measures autistic traits similarly, and the reliability was acceptable and good in the general population of Malaysia and the Netherlands, respectively. However, Malaysians scored higher than Dutch participants. Moreover, 11 AQ-28 items showed cultural bias, indicating that these items are answered/interpreted differently in Malaysia and the Netherlands. Cross-cultural differences in interpreting, reporting, and/or expressing autistic traits highlighted in this study could potentially explain why some items are culturally biased and why Malaysians score higher on these items. The findings of this work imply that cutoff scores derived from one culture should not be generalised to another culture. Moreover, the findings are informative for future development of culturally neutral or appropriate screening and diagnostic tools for autism.