

Report No.: 13922
Date: 08.08.2012
Contact: MB

Task:

Application field: Agriculture

Material: **Horse Bone:**
1) Horse Tibia;
2) Horse teeth bone
3) Horse Mandibula (lower chin bone)

Feed size: 30-50 mm (original sample)
 approx. 1 - 8 mm after pre-cutting in Cutting Mill SM 200

Feed quantity: Complete sample in SM 200
 5 g in CryoMill and Mixer Mill

Material specification(s): dry, brittle

Customer requirements(s): Fine enough for DNA-extraction (usually about < 50 µm)

Subsequent analysis: DNA Deoxyribo Nucleic Acid

Solution

Selected Instrument(s): Cutting Mill SM 200
 CryoMill
 Mixer Mill MM 400

Configuration(s) Item nos.:

- 1 x MM 400, 100-240 V, 50/60 Hz
- 2 x Grinding jar, stainless steel, 25 ml, screw top design
- 2 x Grinding ball, stainless steel, 15 mm ø
- 1 x Cryo kit for cooling the grinding jars with liquid nitrogen
- optional:
- 1 x CryoMill, 100-240 V, 50/60 Hz
- 1 x Grinding jar, stainless steel, 25 ml
- 1 x Grinding ball, stainless steel, 20 mm ø
- 1 x Autofill with LN2 container and safety valve, 50 litres
- 1 x SM 200, 3/N~ 400 V, 50 Hz, cutting bars stainless steel
- 1 x 6-disc rotor, stainless steel, with reversible cutting tips of tungsten carbide
- 1 x Universal hopper with plastic plunger, for SM 200 / SM 300
- 1 x Bottom sieve, square holes, 8 mm, stainless steel

Please note: Other electrical versions of the instrument(s) are available with different item numbers.

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Parameter(s):	SM 200: Revolution speed 1500 rpm MM 400: Frequency 30 Hz CryoMill: Frequency 25 Hz; 8 cycles
Time:	Sample 1, MM 400: 4x 2min. (+ approx. 1 min. intermediate cooling of the jar in liquid nitrogen) Sample 1, CryoMill: 8x 2 min. (automatic pre-cooling, 45s intermediate cooling) Sample 2, CryoMill: 8x 2 min. (automatic pre-cooling, 45s intermediate cooling)
Achieved result(s):	See Horiba-reports (after using ultra-sonic, without ultra-sonic particles up to approx. 100 µm can be measured): Sample 1, MM 400: D90 = approx. 45 µm Sample 1, CryoMill: D90 = approx. 19 µm Sample 2, CryoMill: D90 = approx. 7 µm Sample 3, CryoMill: D90 = approx. 10 µm

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Remark(s):

Pre-cutting of all samples in the Cutting Mill SM 200 (with hinged hopper) with a 8 mm bottom sieve.
Sample could also be cut manually down to < 10 mm.

Sample 1, MM 400, test 1:

Grinding of 5 g sample with the Mixer Mill MM 400 in a 25 ml grinding jar with 1x 20 mm grinding ball of stainless steel (without cooling the grinding jar in liquid nitrogen). The material starts caking and agglomerating (sticking to the grinding jar wall and the ball).

Sample 1, MM 400, test 2:

Grinding of 5 g sample with the Mixer Mill MM 400 in a 25 ml grinding jar with 1x 15 mm grinding ball of stainless steel. In order to improve the breaking properties of the material and reduce the agglomeration and caking effects the filled grinding jar has been cooled in liquid nitrogen (in KryoKit). After every 1:30 or 2 min. grinding time the grinding jar should be cooled again in liquid nitrogen until it stops boiling.

After 4x 2 min. the particle size has been measured. Still a little bit of sample is caking and agglomerating.

Sample 1, CryoMill:

Grinding of 5 g sample with the CryoMill in a 25 ml grinding jar with 1x 20 mm grinding ball of stainless steel.

Due to the fact that the sample is cooled/pre-embrittled continuously in the CryoMill a higher fineness can be achieved.

All parameters can easily be adjusted at the machine. The user has no direct contact with liquid nitrogen.

After 8 cycles of 2 min each the particle size has been measured with the Horiba LA-950.

Sample 2, CryoMill:

Grinding of 5 g sample with the CryoMill in a 25 ml grinding jar with 1x 20 mm grinding ball of stainless steel.

Due to the fact that the sample is cooled/pre-embrittled continuously in the CryoMill a higher fineness can be achieved.

All parameters can easily be adjusted at the machine. The user has no direct contact with liquid nitrogen.

After 8 cycles of 2 min each the particle size has been measured with the Horiba LA-950.

Sample 3, CryoMill:

Grinding of 5 g sample with the CryoMill in a 25 ml grinding jar with 1x 20 mm grinding ball of stainless steel.

Due to the fact that the sample is cooled/pre-embrittled continuously in the CryoMill a higher fineness can be achieved.

All parameters can easily be adjusted at the machine. The user has no direct contact with liquid nitrogen.

After 8 cycles of 2 min each the particle size has been measured with the Horiba LA-950.

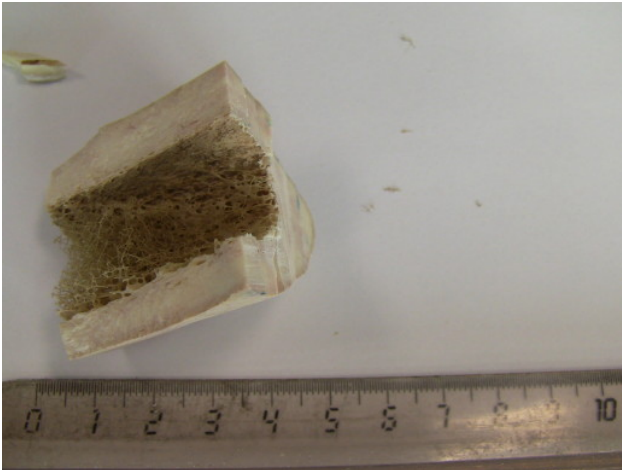
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Without using ultra-sonic during measuring the particle size some agglomerates up to 100 µm are noticeable.
After using ultra-sonic the agglomerates are destroyed and the "real" particle size could be measured (see "Achieved results")

Recommendation: The CryoMill and the Mixer Mill MM 400 are suitable to grind the sample materials under the above mentioned conditions.

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Pictures of the sample:



Picture 1: Sample 1



Picture 2: Sample 1 after pre-cutting in SM 200



Picture 3: Sample 2



Picture 4: Sample 2 after pre-cutting in SM 200

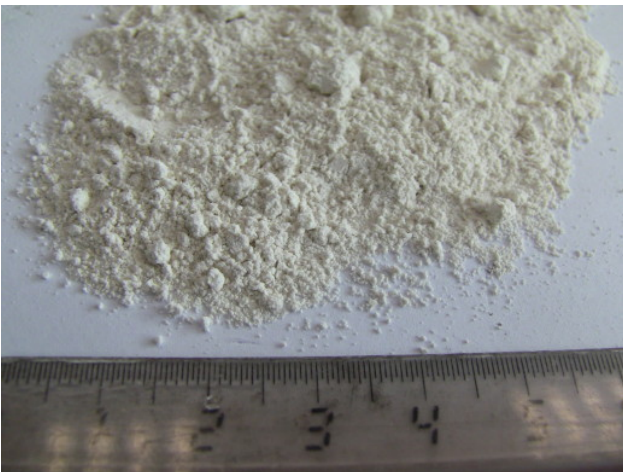
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Picture 5: Sample 3



Picture 6: Sample 3 after pre-cutting in SM 200



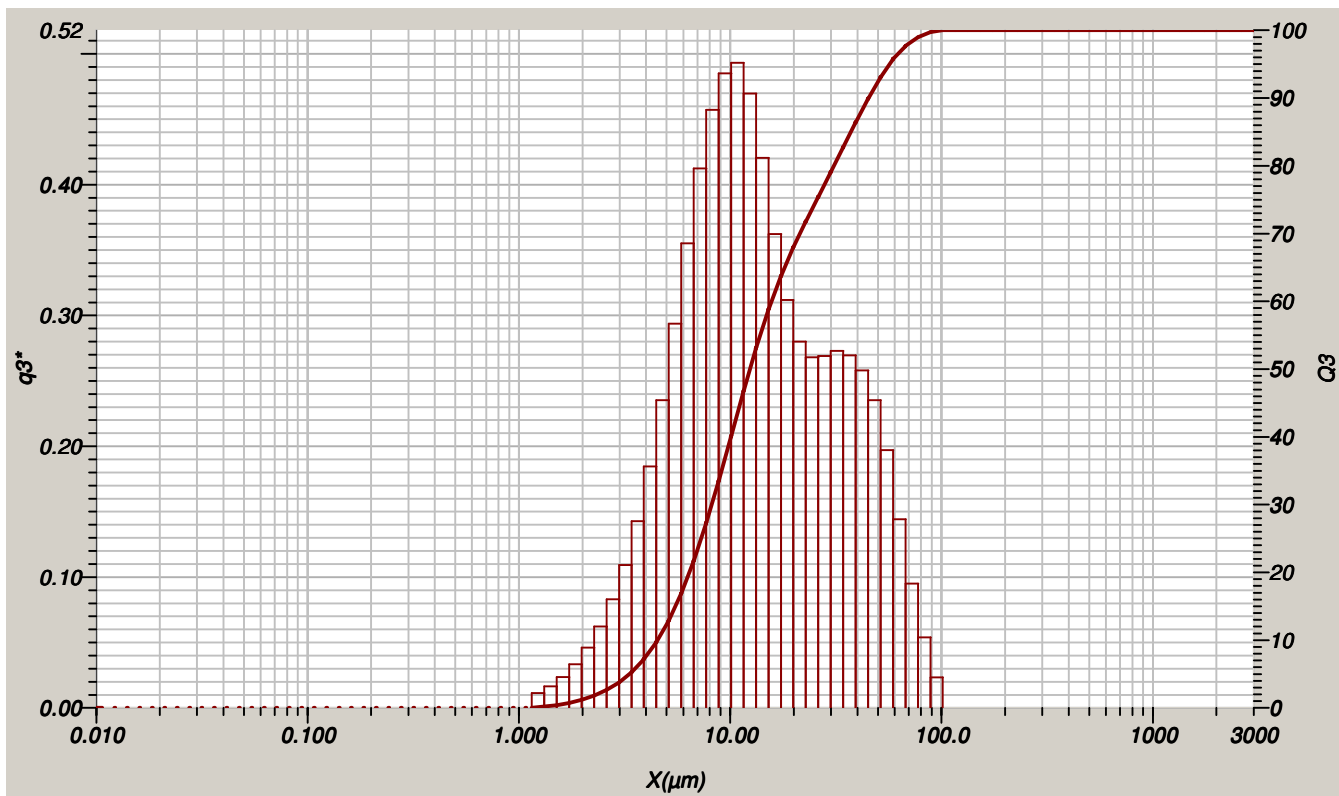
Picture 7: Samples after fine-grinding in CryoMill



Laser Scattering Particle Size Distribution Analyzer LA-950

ID# : 201208071514837
 Probenname : 13922_Horse Tibia_MM400_25mlss_15mmball_4x2min
 Datenbezeichnung : 13922_Horse talia_MM400_25mlss_15mmball_4x2min
 Quelle :
 Transmission (R) : 88.2(%)
 Transmission (B) : 86.4(%)
 Zirkulationsgeschwindigkeit : 6
 Rührgeschwindigkeit : 6
 Ultraschall : 01:00 (7)
 Art der Verteilung : Volumen

D10 : 4.54043(μm)
 Median : 12.40756(μm)
 D90 : 45.22232(μm)



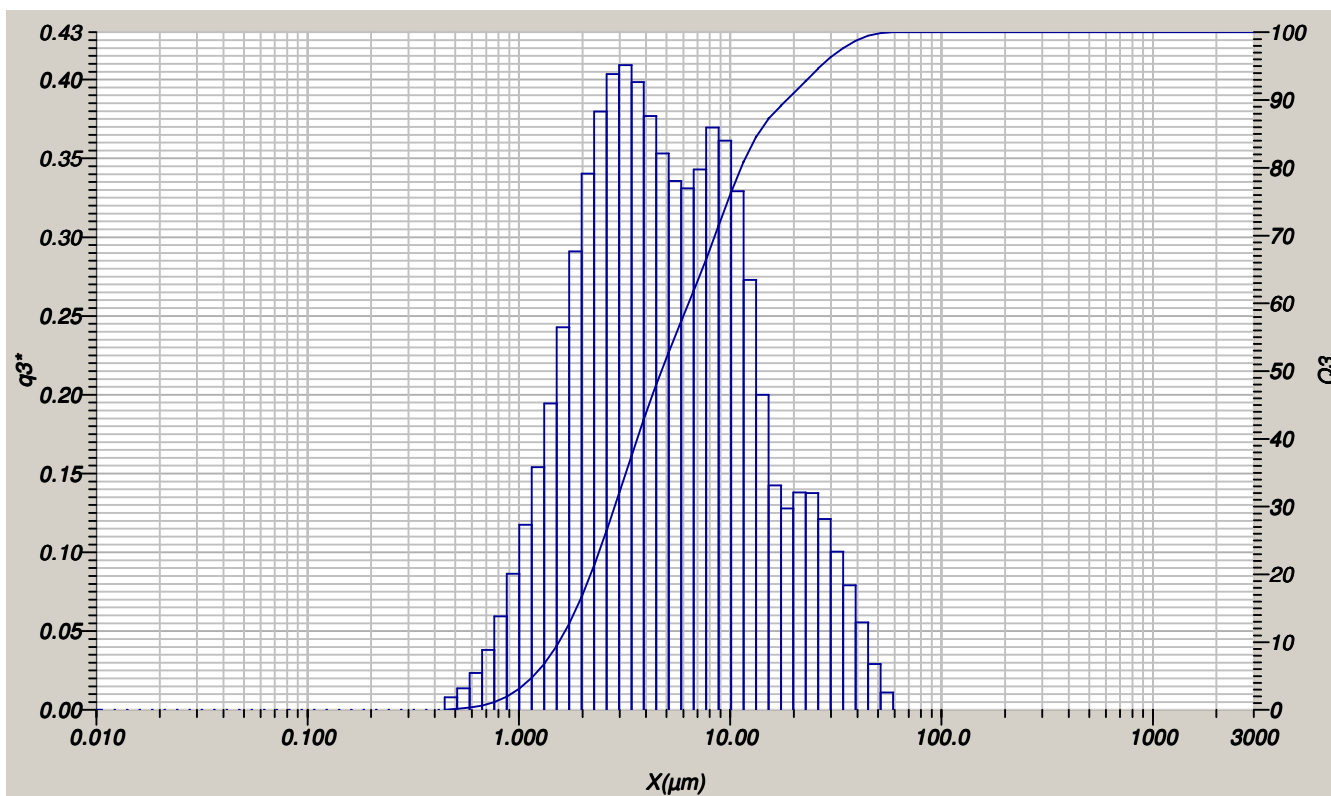
Nr.	X(μm)	q3*	Q3	Nr.	X(μm)	q3*	Q3	Nr.	X(μm)	q3*	Q3	Nr.	X(μm)	q3*	Q3	Nr.	X(μm)	q3*	Q3
35	1.151	0.000	0.000	43	3.409	0.109	5.208	51	10.097	0.485	40.010	59	29.907	0.269	78.996	67	88.583	0.054	99.688
36	1.318	0.011	0.151	44	3.905	0.142	7.142	52	11.565	0.493	46.699	60	34.255	0.273	82.698	68	101.460	0.023	100.000
37	1.510	0.016	0.372	45	4.472	0.184	9.644	53	13.246	0.469	53.070	61	39.234	0.269	86.352	69	116.210	0.000	100.000
38	1.729	0.023	0.688	46	5.122	0.235	12.835	54	15.172	0.420	58.774	62	44.938	0.258	89.852				
39	1.981	0.033	1.138	47	5.867	0.293	16.817	55	17.377	0.362	63.687	63	51.471	0.235	93.043				
40	2.269	0.046	1.761	48	6.720	0.355	21.633	56	19.904	0.312	67.917	64	58.953	0.197	95.715				
41	2.599	0.062	2.604	49	7.697	0.412	27.227	57	22.797	0.280	71.714	65	67.523	0.144	97.670				
42	2.976	0.083	3.727	50	8.816	0.457	33.431	58	26.111	0.268	75.348	66	77.339	0.095	98.958				



Laser Scattering Particle Size Distribution Analyzer LA-950

ID# : 201208080900838
 Probenname : 13922_Horse Tibia_CryoMill_25mlss_20mmball_8x2min
 Datenbezeichnung : 13922_Horse Tibia_CryoMill_25mlss_20mmball_8x2min
 Quelle :
 Transmission (R) : 87.4(%)
 Transmission (B) : 82.7(%)
 Zirkulationsgeschwindigkeit : 6
 Rührgeschwindigkeit : 6
 Ultraschall : 02:00 (7)
 Art der Verteilung : Volumen

D10 : 1.54701(μm)
 Median : 4.73807(μm)
 D90 : 18.55172(μm)



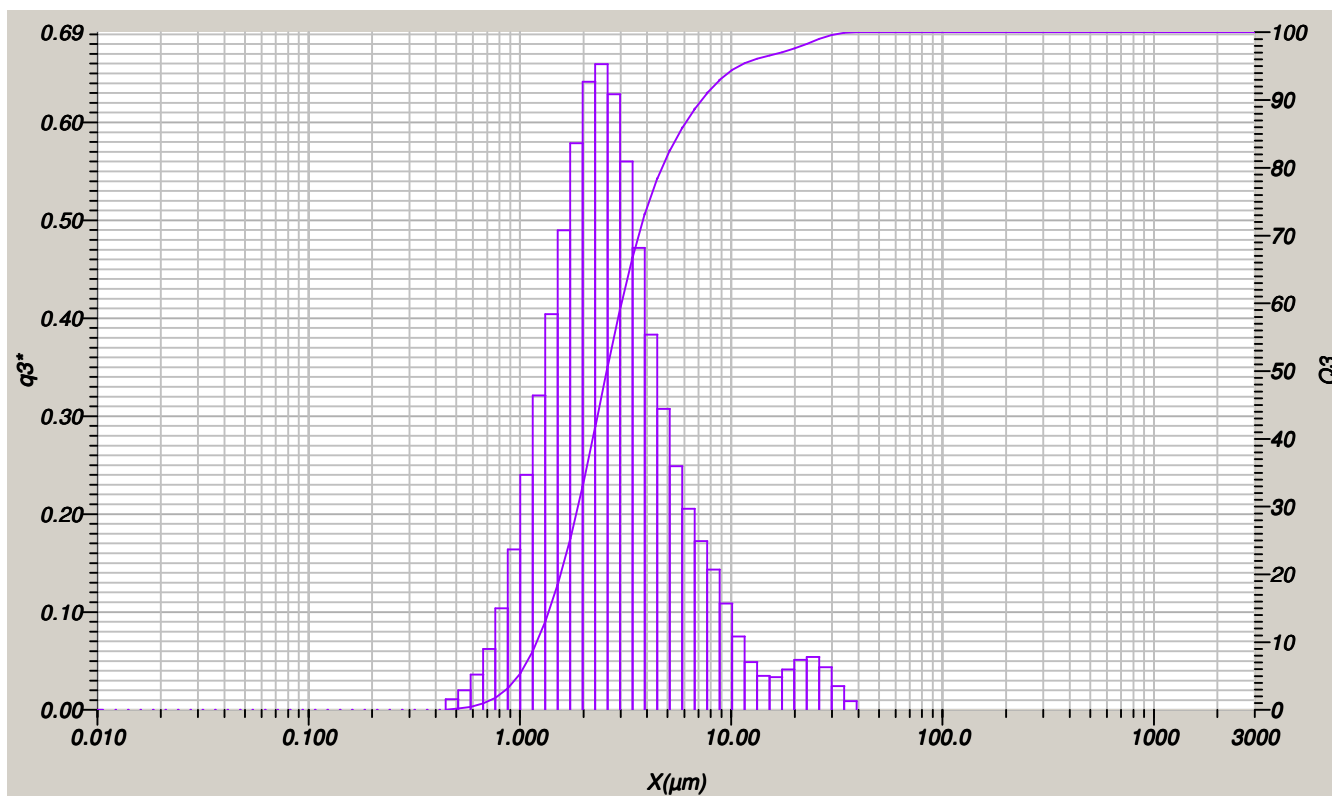
Nr.	X(μm)	q3*	Q3	Nr.	X(μm)	q3*	Q3	Nr.	X(μm)	q3*	Q3	Nr.	X(μm)	q3*	Q3
28	0.445	0.000	0.000	36	1.318	0.154	6.774	44	3.905	0.398	42.848	52	11.565	0.329	80.824
29	0.510	0.008	0.106	37	1.510	0.194	9.411	45	4.472	0.377	47.961	53	13.246	0.273	84.524
30	0.584	0.014	0.290	38	1.729	0.243	12.703	46	5.122	0.353	52.752	54	15.172	0.200	87.235
31	0.669	0.023	0.605	39	1.981	0.291	16.650	47	5.867	0.335	57.304	55	17.377	0.142	89.165
32	0.766	0.038	1.120	40	2.269	0.340	21.267	48	6.720	0.331	61.793	56	19.904	0.128	90.898
33	0.877	0.059	1.922	41	2.599	0.379	26.418	49	7.697	0.343	66.444	57	22.797	0.138	92.768
34	1.005	0.086	3.093	42	2.976	0.403	31.892	50	8.816	0.369	71.459	58	26.111	0.137	94.633
35	1.151	0.117	4.686	43	3.409	0.409	37.443	51	10.097	0.361	76.358	59	29.907	0.121	96.275
												60	34.255	0.100	97.637
												61	39.234	0.079	98.707
												62	44.938	0.055	99.457
												63	51.471	0.029	99.851
												64	58.953	0.011	100.000
												65	67.523	0.000	100.000



Laser Scattering Particle Size Distribution Analyzer LA-950

ID# : 201208080948840
 Probenname : 13922_Horse teeth bone_CryoMill_25mlss_20mmball_8x2min
 Datenbezeichnung : 13922_Horse teeth bone_CryoMill_25mlss_20mmball_8x2min
 Quelle :
 Transmission (R) : 87.3(%)
 Transmission (B) : 82.0(%)
 Zirkulationsgeschwindigkeit : 6
 Rührgeschwindigkeit : 6
 Ultraschall : 02:00 (7)
 Art der Verteilung : Volumen

D10 : 1.20147(μm)
 Median : 2.57494(μm)
 D90 : 7.26641(μm)



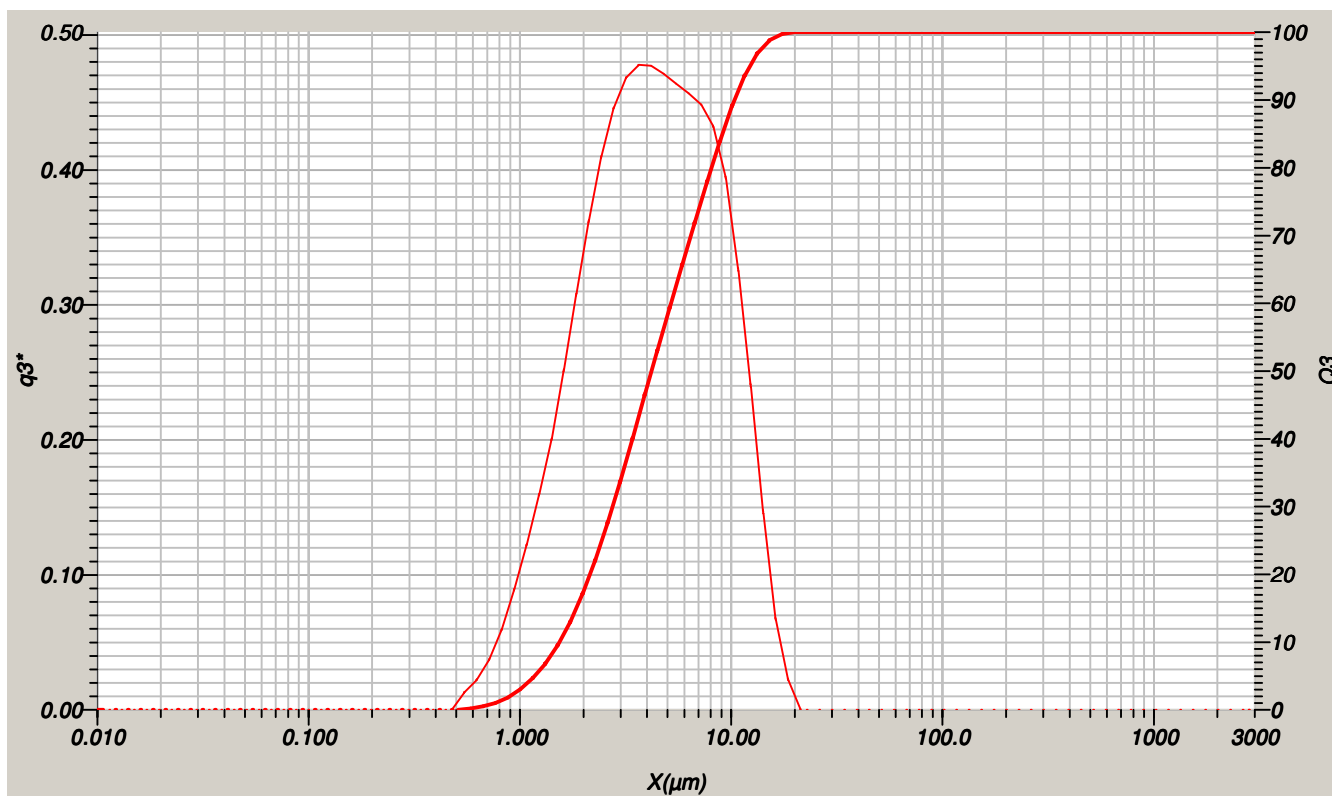
Nr.	X(μm)	q3*	Q3	Nr.	X(μm)	q3*	Q3	Nr.	X(μm)	q3*	Q3	Nr.	X(μm)	q3*	Q3				
28	0.445	0.000	0.000	36	1.318	0.321	12.974	44	3.905	0.471	73.128	52	11.565	0.075	95.415	60	34.255	0.024	99.883
29	0.510	0.011	0.145	37	1.510	0.404	18.457	45	4.472	0.383	78.327	53	13.246	0.048	96.072	61	39.234	0.009	100.000
30	0.584	0.020	0.414	38	1.729	0.490	25.101	46	5.122	0.307	82.497	54	15.172	0.035	96.541	62	44.938	0.000	100.000
31	0.669	0.036	0.899	39	1.981	0.578	32.952	47	5.867	0.249	85.870	55	17.377	0.033	96.993				
32	0.766	0.062	1.740	40	2.269	0.641	41.656	48	6.720	0.205	88.654	56	19.904	0.041	97.549				
33	0.877	0.104	3.146	41	2.599	0.659	50.601	49	7.697	0.172	90.990	57	22.797	0.051	98.239				
34	1.005	0.164	5.366	42	2.976	0.628	59.131	50	8.816	0.143	92.931	58	26.111	0.054	98.970				
35	1.151	0.240	8.622	43	3.409	0.560	66.729	51	10.097	0.108	94.403	59	29.907	0.043	99.557				



Laser Scattering Particle Size Distribution Analyzer LA-950

ID# : 201208081153861
 Probenname : 13922_Horse Mandibula_CryoMill_25mlss_20mmball_8x2min
 Datenbezeichnung : 13922_Horse Mandibula_CryoMill_25mlss_20mmball_8x2min
 Quelle :
 Transmission (R) : 85.6(%)
 Transmission (B) : 79.7(%)
 Zirkulationsgeschwindigkeit : 6
 Rührgeschwindigkeit : 6
 Ultraschall : 02:00 (7)
 Art der Verteilung : Volumen

D10 : 1.53918(μm)
 Median : 4.20583(μm)
 D90 : 10.37547(μm)



Nr.	X(μm)	q3*	Q3	Nr.	X(μm)	q3*	Q3	Nr.	X(μm)	q3*	Q3	Nr.	X(μm)	q3*	Q3
29	0.510	0.000	0.000	37	1.510	0.200	9.517	45	4.472	0.477	52.929	53	13.246	0.240	96.802
30	0.584	0.013	0.173	38	1.729	0.251	12.928	46	5.122	0.471	59.322	54	15.172	0.146	98.789
31	0.669	0.022	0.475	39	1.981	0.307	17.100	47	5.867	0.464	65.615	55	17.377	0.067	99.700
32	0.766	0.037	0.979	40	2.269	0.362	22.010	48	6.720	0.457	71.812	56	19.904	0.022	100.000
33	0.877	0.059	1.781	41	2.599	0.409	27.567	49	7.697	0.448	77.896	57	22.797	0.000	100.000
34	1.005	0.089	2.985	42	2.976	0.446	33.615	50	8.816	0.432	83.761				
35	1.151	0.122	4.640	43	3.409	0.468	39.971	51	10.097	0.394	89.113				
36	1.318	0.159	6.801	44	3.905	0.478	46.454	52	11.565	0.326	93.540				